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Recommendations for microbiological sampling and contamination tracer use aboard the *JOIDES Resolution* following 20 years of IODP deep biosphere research

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Introduction

If you are reading this, you may be thinking about sailing, will sail in the near future, or are currently sailing as a microbiologist on an International Ocean Discovery Program (IODP) expedition implemented by the *JOIDES Resolution* Science Operator (JRSO). This is a life changing decision and will be well worth your effort. There are many aspects of conducting microbiology research on the research vessel (R/V) *JOIDES Resolution* that are unique; therefore, this *Technical Note* should serve as a guide to help you make decisions that will ensure you are using best practices and enable you to collect the best possible data. This note is also useful for those who are interested in how contamination testing is performed on board *JOIDES Resolution*, as well as anyone considering requesting microbiological samples from a *JOIDES Resolution* expedition.

This publication is a follow-up to Ocean Drilling Program (ODP) *Technical Note* 28 (Smith et al., 2000), which laid out the original procedures for contamination control when processing samples for microbiology aboard *JOIDES Resolution*. Significant advances in sampling techniques, nucleic acid sequencing, and activity assays, among other areas, have led to changes in best practices for microbiology within IODP and on board *JOIDES Resolution*. A current synthesis is presented here. Likewise, we have gained 20 years worth of data and experience in deploying microbiological contamination tracers. This insight allows us to evaluate their utility and better identify the most common sources of contamination during coring and drilling. We conclude that fluorescent microspheres, as currently deployed, are not a reliable or effective means of tracing contamination and recommend ending their use. We further outline shipboard objectives and expectations management as a function of the number of sailing microbiologists and provide guidance on reasonable shore-based requests for samples.

This *Technical Note* originated from discussions during a small workshop of expert users and JRSO staff held at the JRSO in 2017 and a period of solicited community input (see **Acknowledgments**); it therefore reflects broader input from the deep biosphere community than is reflected in the author list. Although the practices and suggestions laid out here are not binding, they represent best practices for microbiologists based on the current capabilities of *JOIDES Resolution*, specifically, as of 2020. Users interested in microbiology research on the drilling vessel (D/V) *Chikyu* can find information at the Kochi Core Center Deep Biosphere Samples web page (**http://www.kochi-core.jp/DeepBIOS**). Mission-specific platform (MSP) microbiology research is bespoke to each project, but this guide, in tandem with the *Chikyu* guide, can be helpful for constraining what is possible.

Coring types and tracer runs: how contamination testing is currently implemented on board *JOIDES Resolution*

Coring systems and how they impact potential contamination

Three coring systems are used on board *JOIDES Resolution*, the advanced piston corer (APC), extended core barrel (XCB), and rotary core barrel (RCB). Full details on these systems, as well as schematics, can be found at **http://iodp.tamu.edu/tools/index.html**. Simplified diagrams of all three systems are provided in Figure **F1**.

Broadly speaking, the APC system is used for soft sediments. Cores recovered with this system are denoted by the suffix "H" or "F" directly after the core number in a sample's name. Once the material becomes too firm for piston coring, the XCB system is generally deployed (cores denoted by the suffix "X"). The RCB system is used for basement and any sedimentary rock too difficult to recover with the XCB system. Cores collected using the RCB system are denoted by the suffix "R".

APC and XCB coring

The APC system is the most common coring system used for collecting sediments and is usually the first system deployed when an expedition arrives at a site. It also produces sediment cores with the least amount of visual disturbance relative to the other coring systems, and therefore has the lowest likelihood of microbiological contamination when coring soft to semiconsolidated sediments. When sediments are recovered without disruption, they generally have a lower potential for contamination from seawater circulated in the borehole and/or drilling mud (House et al., 2003; Lever et al., 2006; Sauvage et al., 2017). In addition to a lower likelihood of contamination during drilling, piston-cored sediments can easily be sampled after sectioning on the catwalk from the center of the whole-round (WR) sediment section (i.e., no contact with the plastic core liner), where the likelihood for contamination is lower.

Once using the APC system is no longer possible, including the implementation of the half-length APC system, where the APC system is advanced at a half stroke instead of a full stroke (resulting in about a 4.5 m long core as opposed to a 9.5 m long core), many expeditions switch to the XCB system. The XCB system targets medium to hard sediments—essentially material that is too hard for piston coring but may be too soft to recover well with the RCB system. One major benefit of the XCB system is that it uses the same bottom-hole assembly as the APC system, which means that there is no time penalty for switching between the APC and XCB systems (in contrast to moving to the RCB system). A benefit of the XCB system specific to microbiology sampling is that mud sweeps (introduction of heavy mud into the borehole; see **RCB coring**) are generally not performed, which thus eliminates that particular source of contamination. However, the XCB system arguably produces the worst core quality with the highest likelihood of disturbance; "biscuiting" is particularly common (Figure **F2**). Studies of microbial contamination during drilling have found that the XCB system yields a higher potential for contamination than the APC system (House et al., 2003; Lever et al., 2006).

In cores recovered with the APC and XCB systems, it is common practice to collect syringe/spatula samples or WR sections immediately adjacent to where WRs are taken for interstitial water (IW; i.e., pore water) analyses. This allows for the integration of microbiology and geochemistry analysis and results because both samples come from nearly the same depth. In selecting WRs for sampling, avoid areas with biscuiting (Figure **F2**). Biscuiting occurs when cored material fragments into discrete chunks, or "biscuits." Slurry, a mixture of drilling fluid, seawater, and ground sediment, can be injected between and around the biscuits, producing what is colloquially termed "biscuits and gravy." This disturbance type can introduce microbial contamination and can be difficult in some cases to identify visually. The recently acquired X-ray imaging capabilities on *JOIDES Resolution* allow for imaging of core sections prior to splitting and selection of the "best" microbiological WR but does add significant time to the sampling process. Pearson and

Nicholas (2014), as well as the review by Jutzeler et al. (2014), provide some images and extensive discussion of visual disturbance, including biscuiting, fall-in textures (sediment from higher up in the hole falls to the bottom of the borehole and is recovered in a core), uparching bed contacts, flowage along the core liner (evidenced by a thin line of sediments closest to the core liner different from those in the center, which may be difficult to determine for a WR sample prior to splitting; more common in APC cores), occurrence of APC partial strokes, and liquefaction (again, more common in APC cores; Figure **F2**).

RCB coring

The RCB system is deployed once material becomes too indurated for the XCB system or when coring igneous basement. Fracturing, fragmentation, and brecciation as a result of the rotary coring process are common drilling-induced disturbances that can be produced with the RCB system. When coring with the RCB system, it is necessary to periodically conduct "mud sweeps." During this procedure, a more viscous or heavy mud mixture (containing sepiolite and/or barite to increase its density) is pumped down through the drill pipe to the bottom of the borehole to wash accumulated material ("cuttings") out of the hole and onto the seafloor. This mud sweep has the potential to introduce microbial contamination, so it is important to know when these sweeps are conducted. Mud sweep frequency depends on the condition of the borehole but can occur as often as every core. In general, there is likely to be less contamination just before a mud sweep than during the sweep and during a period of recovery following the mud sweep. To aid in identifying possible contamination from mud sweeps, it is possible to make a request to the JRSO Operations Superintendent, Curator, and/or Expedition Project Manager (EPM) for a sample of the drilling mud used during the expedition. Mud sweeps conducted during coring on *JOIDES Resolution* are documented in the RCB cores drilling summary available under the Drilling Reports tab under Summaries in the JRSO's Laboratory Information Management System (available at **http://web.iodp.tamu.edu/LORE**). Older data can be found on the Janus server (see **https://web.iodp.tamu.edu/janusweb/links/links_all.shtml**).

In general, the quality and quantity of the core recovered from basement via RCB coring highly depends on the nature of the material being drilled. For example, in highly fractured systems, it is not uncommon to recover <30% of the length of the core advance, in many separate pieces (Figure **F2**). Some of these pieces, referred to as "rollers," can move around the core liner, making it extremely difficult to determine original orientation and also exposing them to seawater and drilling mud on all sides. They should be avoided for microbiology sampling because of the higher likelihood of contamination and also because once the outer portions of the sample are removed to limit contamination, there is not likely to be enough sample left to generate results.

During some expeditions where microbiological sampling is a top priority, RCB cores are brought from the catwalk into the core splitting laboratory and "shaken" out into split core liners for rapid initial inspection and selection of WRs for microbiological sampling. During this stage, only the core technicians and the lead petrologist and microbiologist on shift touch the material, wearing appropriate personal protective equipment (clean nitrile gloves at a minimum, preferably with a mask as well, as done during IODP Expeditions 360 and 385) to prevent contamination of the core exteriors. Once representative pieces are identified for microbiological sampling, these pieces are photographed before being removed from the core by the lead microbiologist on shift using sterilized implements (e.g., combusted aluminum [Al] foil or tongs) and transferred to the microbiology laboratory for further processing. A Styrofoam insert is placed in the core where the WR was removed, and the core is then run through scanning tracks and the rest of the traditional core flow. The science party may choose to first run the intact core section through the new X-ray imaging track on *JOIDES Resolution* before deciding which WR piece(s) to remove. During expeditions where microbiology is not a major objective, microbiologists on board should expect that the core sections may be run through all core scanning tracks before being opened, which can take several hours.

These considerations about which coring methods may cause which specific sample disturbances are likely more important when (1) fewer microbiologists are on board (which makes running tracers difficult), (2) tracer has not been run the entire time during coring, or (3) shore-based scientists may be looking at core photos to help decide where to request a sample. Shore-based sampling parties for microbiology must also consider this, as well as considerations about sample preparation, discussed in **Expectation management for scientists and JRSO staff**.

Contaminant tracers and deployment methods

Contamination tracer experiments on board *JOIDES Resolution* involve the introduction of chemical and/or particulate tracers during coring and then their quantification after recovering core material. These tracers have been used while coring unconsolidated sediments with the APC system, more consolidated sediments with the XCB system, and sedimentary and igneous rock using the RCB system. A successful tracer deployment demonstrates that it was delivered to the outside of the sample (e.g., in the core liner) but did not penetrate into the interior, which will actually be used for microbiological sampling and experiments. Detailed descriptions of the original chemical tracer used (perfluoromethylcyclohexane [PMCH], a perfluorocarbon compound) and the fluorescent microsphere particulate tracer can be found in Smith et al. (2000).

Deployment of perfluorocarbon tracers and subsequent sampling

Perfluorocarbon compounds were initially proposed as a tracer because they are chemically inert and can be measured with high sensitivity across several orders of magnitude of dilution (Kallmeyer, 2017), making them effective tracers of water intrusion into the inner parts of cores. Their successful use as a tracer in terrestrial drilling projects led to their adaptation on *JOIDES Resolution*. Although it has been successfully deployed during numerous expeditions, the PMCH molecule is extremely volatile, especially when heated, and once a few samples have been processed in the laboratory, volatilized tracer subsequently builds up in the air in the laboratory (Orcutt et al., 2017; Sauvage et al., 2017). Likewise, extreme care needs to be taken when diluting the concentrated compound for injection into the borehole with drilling fluids. More recently, *JOIDES Resolution* has experimented with the use of perfluoromethyldecalin (PFMD), an equally inert and sensitive but less volatile alternative to PMCH. As of the writing of this publication, PFMD tracers have been used during IODP Expeditions 360, 366, 376, and 385.

Regardless of which perfluorocarbon tracer (PFT) is preferred, microbiologists that want chemical tracers to be used during coring need to inform the EPM of this request as early as possible in the cruise planning process so that it can be balanced against

other expedition objectives. PFTs also need to be ordered far in advance, requiring early coordination between shipboard microbiologists (if more than one) as to which compound and what quantities will be used. These conversations can be facilitated by the EPM. A common strategy for sediment-focused expeditions with major microbiology objectives is to core a dedicated hole for microbiological sampling and to only run PFTs during operations in this dedicated hole so that they are used in a targeted and cost-efficient manner.

Prior to the initiation of coring, PFTs are introduced from a carboy to the drilling fluid at a consistent rate by a high-performance liquid chromatography pump connected to the drilling mud stream through a valve that delivers the drilling mud stream. PFTs are then pumped down the borehole with the circulating seawater and/or drilling mud. This operation is implemented by the driller and other members of the rig crew and needs to be requested by a scientist through either the Co-Chief Scientists or EPM on board well in advance of coring. Sufficient time needs to be allowed for the compounds to reach the coring shoe/bit before coring commences.

Typically, the PFTs are injected in their pure form as delivered from the manufacturer because the compounds have very low solubility in water. With *JOIDES Resolution*, it is not possible to know the exact concentration of the PFT in drilling mud before it leaves the ship because it is likely still dissolving into solution after injection in the mud pump room. To get an approximate concentration, it is possible to collect samples of the drilling mud on the rig floor before the mud is injected into the drill string, but this requires advance coordination through the EPM and Operations Superintendent. This sampling should be done sparingly and should not be considered routine. The presence of PFTs on the exterior of the core is a better metric of successful delivery.

Once a sediment core is recovered and sectioned on the catwalk, samples are collected to detect the presence of PFTs in the core. Two cut-end syringe plugs are taken from the bottom of a section (sections are typically 1.5 m long), one plug is taken from the outer edge of the same section (near the core liner) and the other is taken from the center of the section. The sample should not be taken from the first section of the core, which may contain fall-in material that impacts the results. If the sediment is too indurated to use a syringe, other tools must be used. These tools should be passed through the flame of a torch to remove any PFTs. The boiling point of PFMD is 160°C; therefore, tools need to be heated well over that temperature. If possible, torching the tools should be done on the catwalk. However, it is important to note torching can release vapors of both PMCH and PFMD if the flame is too close to a PFT source (core sections); therefore, where the torching is done is important and must be considered to avoid false positives. Once sampled, exterior and interior samples from the core are then pared away. The samples are placed in headspace vials and immediately sealed. Blank control samples should also be taken on the catwalk and in the laboratory to account for tracer volatilization by filling a headspace vial with an aliquot of water and then sealing. Because the exterior of the core liner is assumed to be coated with drilling fluid, contact with the liner should be avoided while collecting samples. Details of sampling PFTs from sediment cores are provided in Smith et al. (2000), House et al. (2003), Lever et al. (2006), and Sauvage et al. (2017).

The procedure for any cores curated as "hard rock" (referring to hard sedimentary or igneous rocks) is slightly different; as described above (see **RCB coring**), these cores are not sectioned on the catwalk. Several sampling options are available for analysis of the presence of PFTs, but the minimum recommendation is to collect the following samples:

1. A sample from the outside of the selected core piece,
2. Chunks from the inside of the same piece after the cores are split with sterile tools,
3. Small pieces found loose in the core liner because these should have been exposed to PFTs in the seawater pumped down the drill pipe and circulating in the borehole, and
4. Any fluid remaining in the liner, which can also be considered a positive control because it should contain dissolved tracer if PFTs were circulating during coring.

Several small pieces of rock for each sample type (1–3) should be weighed first and then placed directly into headspace vials and immediately sealed. This way, one can standardize the quantified PFT to grams of rock. It is recommended to try to sample similar weights of rock for each measurement. For liquid samples (4), the volume of sample should be standardized. Alternatively, a cotton swab can be used to wipe the interior of the core liner that is then placed in a headspace vial and sealed (ensure some clean Milli-Q water is added to aid volatilization).

Ideally, PFTs are removed from the rock surface prior to sampling the interior to prevent the transfer of the tracer into the interior during sample preparation. This can be accomplished by rinsing the exterior of the rock sample(s) with water or methanol and then drying it under a flame or exposed to air in a clean environment, such as a positive pressure space or a KOACH system (Figure **F3**). For flaming, the piece should be held with tongs under the flame from a handheld propane torch until it appears dry. Initial experiments conducted during ODP Leg 185 showed that drying the surface of the rock with a flame was the best method. However, there is a concern that this may compromise the usefulness of the sample for subsequent microbiological and/or geochemical analyses because of the heat, desiccation, and/or conversion of biomass to char. Further assessment during Expedition 360 indicated that rinsing the samples three times with sterile seawater in unused plastic bags followed by spraying the exterior with 70% ethanol and allowing it to evaporate before sampling was equally effective (MacLeod et al., 2017). After removing PFTs from the exterior, pieces from the interior of the rock are obtained by paring away the exterior using a flamed hammer and chisel while the rock is held on a sheet of Al foil. After each paring, the tools are cleaned of PFTs by passing them through the torch flame, and the rock is placed on new Al foil sheets. When the entire exterior of the rock is removed, the residual rock (interior) is placed in a percussion mortar and crushed with a pestle or further broken down with the hammer and chisel. Again, it is very important to also collect blank background samples of the laboratory air before and after sample handling to account for increased concentrations of PFTs from volatilization of tracer on tools and sample exteriors (Orcutt et al., 2017).

As of the writing of this document, PFTs, and specifically the less volatile PFMDs, are the best means for tracing potential microbial contamination on *JOIDES Resolution*. As outlined below, the number of dedicated sailing microbiologists and mission objectives will impact the ability to run PFTs consistently during coring. We recommend their use for all coring that will result in microbiology sampling when possible.

Deployment of particulate tracers (microspheres)

The only particulate tracer that has been tested and used on *JOIDES Resolution* is fluorescent microspheres (Smith et al., 2000).

These were originally selected for use because they are easy to detect via fluorescence microscopy either during the expedition or during postexpedition research and had been shown to perform well in terrestrial drilling programs (Harvey et al., 1989; Colwell et al., 1992). They are roughly the same size as subseafloor microorganisms and therefore likely to mimic them in terms of their advection or diffusion into sediment or rock pore spaces.

In practice, microspheres are first suspended into solution and ultrasonicated, and then a small volume is transferred into Whirl-Pak bags. These bags are then draped over the mouth of the core catcher sub and taped in that position (Figure **F4**). During coring with the APC system, the core barrel rips through the Whirl-Pak bag, distributing the microspheres. During XCB and RCB coring, the core barrel free falls in the drill string from the drill floor to the bottom of the hole, upon which the Whirl-Pak bag breaks open and the microspheres are delivered into the circulating drilling fluid. This procedure was developed because it proved impractical and cost prohibitive to add microspheres to the entire vat of drilling mud and keep them homogeneously distributed (Kallmeyer, 2017).

There are several disadvantages of this approach. It is difficult to know if the bags exploded as planned, releasing the particles into solution. Likewise, it is uncertain if the microsphere "slurry" is equally distributed along the length of the core. Figure **F4** shows how the bag holding the microspheres can get lodged in the core liner, which contributes to localized dispersal of the microspheres. Especially for RCB coring in basement, it is unlikely that the microsphere slurry maintains a constant concentration during advancement because each core advance generally takes several hours. Finally, sampling in hot environments, such as Integrated Ocean Drilling Program Expedition 331 on *Chikyu*, can result in likely thermal degradation or melting of the microspheres to the point where none can be detected in any of the samples (Yanagawa et al., 2013). Published studies reveal that microspheres are not consistently detected on the exterior of cores, with inconsistent delivery (House et al., 2003) and nondetectable counts occurring up to 21% of the time (House et al., 2003; Yanagawa et al., 2013). All of these can lead to "false negatives," where absence of microsphere tracers is interpreted as a sign of no contamination when in fact the microspheres were never in contact with the sample exterior in the first place. Even when microspheres are detected, their presence can only be recorded in a qualitative sense (i.e., few particles or a lot of particles) and not quantitatively.

For these scientific reasons primarily, but also considering the disproportionate burden on the core technicians and rig crew to implement and deploy them, we do not recommend using microspheres on future expeditions.

Alternative tracer strategies and control samples

In terrestrial drilling, where it is possible to tightly control the delivery of synthetic tracers into drilling muds, fluorescent dyes have been shown to be a cheap and effective alternative to microspheres and PFTs (Kallmeyer, 2017). These dyes have not been tested or implemented on *JOIDES Resolution* to date because fluorescent dyes can fade with light exposure (as they would be when mixed with drilling mud on *JOIDES Resolution*), adsorb to sepiolite used in *JOIDES Resolution* drilling mud, or react with metal ions in a formation's IW (Kallmeyer, 2017). Recently, a new fluorescent particulate tracer was introduced to scientific drilling (Friese et al., 2017). This tracer has similar properties to microspheres but is three to four orders of magnitude less expensive. The sensitivity is in the same range as PFTs. The tracer was successfully employed during several International Continental Drilling Program drilling projects. The lower cost allowed for mixing the tracer directly into the drilling mud, thereby ensuring consistent delivery to the core. However, given the very high flow rates of drilling mud on *JOIDES Resolution* and the open hole coring strategy (all cuttings/mud circulated to the seafloor), even the reduced cost does not make this approach financially feasible as a standard tool for the JRSO. However, it is possible this might still be a suitable tool for the other IODP platforms (e.g., *Chikyu* and MSPs), and further testing would be required.

In some cases, the scientific goals of an expedition may not include microbiology as a primary objective of the planned research and yet there may still be a request for collection of microbiological samples. Unfortunately, in this case, it would be unlikely that a full tracer program oriented toward microbiological sample integrity could be implemented. Nevertheless, with some additional planning, core and control samples can be collected and preserved for subsequent limited microbiological characterization by comparing the microbes present in the cores to those in drilling fluid. This method was first explored during terrestrial coring (Lehman et al., 1995), and because the cost of high-throughput gene sequencing has decreased significantly in the last decade, this option may be even more viable. For such expeditions, WRs (~10 cm in length) may be selected and preserved as requested by the shore-based (or shipboard) microbiology team. At the same time that these WRs are collected, samples of drilling fluid should be obtained from the mud tank and from the core liner when it is cut on the catwalk. These samples, which correspond with given WR(s) from a core, should be preserved (e.g., frozen at −80°C) along with the cores.

Subsequently, microbiologists can analyze the microbial community composition in subcores from the WRs along with drilling fluid samples (mud tank and core liner fluid) collected at the same time. When community composition in the subcores is found to be indistinguishable from drilling fluid, the subcore samples would be deemed contaminated. However, when sufficient differences exist between these two types of samples, the core may not have been contaminated by drilling fluid. This method may be developed even further if indicator taxa are detected that are always associated with drilling fluid (e.g., classic seawater microbes or microbes specifically associated with a drilling fluid additive) but rarely or never associated with deep core material. Enumeration of total cells in both the drilling fluid and in the cores using a sensitive method like droplet digital polymerase chain reaction (PCR) (Hindson et al., 2011) may help establish the degree to which a core has been compromised. Sheik et al. (2018) explored this concept for subsurface samples and provided in-depth suggestions for how to proceed with sample analysis using subtractive analysis with blanks and proper controls.

Postexpedition sampling recommendations

After an expedition, split core archive and working halves, as well as any microbiological WRs collected for archiving, are sent to one of the IODP core repositories for long-term storage. This material can be requested by the scientific community for additional analyses. Here, we discuss the important considerations to keep in mind regarding the use of this material for microbiological sampling.

The IODP maintains three core repositories globally. The Gulf Coast Repository in College Station, Texas (USA), contains cores collected in the Pacific Ocean (east of the Pacific plate western boundary), Caribbean Sea, Gulf of Mexico, and Southern Ocean

south of 60° (except the Kerguelen Plateau). The Bremen Core Repository in Bremen (Germany) houses cores collected in the Atlantic and Arctic Oceans (north of the Bering Strait) and Mediterranean, Black, and Baltic Seas. The Kochi Core Center in Kochi (Japan) maintains cores collected in the Pacific Ocean (west of the Pacific plate western boundary), Indian Ocean, Kerguelen Plateau, and Bering Sea. Further details are available at **https://www.iodp.org/resources/core-repositories**.

The archive and working halves of cores are stored in the repositories at 4°C under ambient air. Thus, this material is generally not suitable for microbiological sampling because changes in the microbial community, and potentially in pore water chemistry, can occur during storage. For example, large changes in community composition and abundance have been documented from samples collected from the same core depths collected at sea and then after storage at 4°C (Mills et al., 2012). Mold and fungi can also grow on the split core faces during storage. Examples of useful microbiological analyses coming from archived core sections stored under these conditions do exist (e.g., Klein et al., 2015), but this will only work when investigating properties or biological signatures that would be unaffected by these storage-induced changes.

Furthermore, it is important to note that all core sections in the repositories have already been split lengthways on *JOIDES Resolution*. APC cores are generally split using a wire and no water; however, where sediment is very clayey, sticks to the wire, and/or becomes more indurated, as for XCB/RCB cores, the cores will have been in contact with water and a splitting saw. In all cases, splitting tools are not kept sterile and may introduce contamination.

In summary, sampling core section halves maintained at 4°C in core repositories for microbiology is strongly discouraged and should be limited to the purposes of identifying organisms that have cellular structures that are preserved in sediments, such as coccolithophores and foraminifers. DNA- or RNA-based analyses and most work targeting analyses of prokaryotic communities or viruses is not recommended.

Beginning with ODP Leg 201 in 2002, archival WR microbiological samples were collected during expeditions with dedicated microbiological objectives, although this type of sampling is still not a standard practice (Orcutt et al., 2014). These archival WRs are stored frozen at −80°C in the repositories and can be requested by the broader scientific community for usage. A list of available IODP data related to all samples can be found at **http://www.iodp.org/resources/access-data-and-samples**, and lists of samples preserved at −80°C can be found at the websites listed in Table **T1**. Each repository may have their own system for sampling from the collection of cores stored at −80°C, and interested parties should consult with the corresponding curator/curatorial staff for further details. These samples are the only ones from the repositories that we recommend for DNA, RNA, cell quantification, or any other analyses that require preservation of the sample at the time of sampling and minimal risk of contamination.

Sampling during a shore-based sampling party

Some expeditions implement postexpedition shore-based sampling parties. For an expedition where a sampling party is planned (detailed in an expedition's *Scientific Prospectus*), it is ideal that microbiologists are still allowed to sample shipboard and freeze WR samples, but this may not be possible. We recommend that any interested scientists contact the EPM for your expedition as soon as possible to discuss shipboard sampling. Compared to samples in the core repositories, which will have sat at 4°C for at least 1 y postexpedition during the moratorium (period of time during which only expedition scientists are allowed access), a shore-based sampling party will happen generally within 6 months postexpedition. Microbiologists sampling from shore-based sample parties should consider all of the aspects discussed above in regard to selecting samples that will have the lowest likelihood of contamination. It is advisable to scrape off the outer edges of samples before collecting, and caution should be taken in interpreting any DNA, RNA, cell count, or other data streams that could have altered during storage. Enrichment culturing to isolate new microorganisms, which is not quantitative, could be done effectively with samples collected in such a way.

Expectation management for scientists and JRSO staff

In this section, we discuss the reasonable implementation of microbiology sampling needs during a *JOIDES Resolution* expedition given the priority of microbiology/microbiological sampling for achieving expedition objectives and the number of shipboard microbiologists. This discussion is summarized in Table **T2**. Please keep in mind that the guidelines/suggestions here, particularly in this section, only applies to *JOIDES Resolution* and the JRSO. Other IODP platforms (*Chikyu* and MSPs) managed by different implementing organizations (Japan Agency for Marine-Earth Science and Technology and European Consortium for Ocean Research Drilling) have different capabilities, procedures, and levels of support available.

Preexpedition planning considerations

It is important for soon-to-be sailing microbiologists, as well as all scientific participants, to understand the challenges and difficulties while operating at sea. These challenges have the potential to affect the amount and quality of core recovered as well as the operational time available itself. After an expedition is scheduled for *JOIDES Resolution*, a call for applications to sail is made and a webinar is scheduled for the Co-Chief Scientists to summarize the expedition goals and generate interest for scientists to sail. Ideally, any interested microbiologist would contact the EPM and/or Co-Chief Scientists once an expedition is scheduled to determine whether microbiology work will be feasible during the expedition so that microbiology can be included in the webinar and expedition *Scientific Prospectus*. The *Scientific Prospectus* is the preliminary plan for an expedition, largely based on the drilling proposal, that outlines planned operations and scientific objectives. Inclusion of microbiology in the *Scientific Prospectus*, even if not in the original proposal, helps ensure the JRSO can obtain the necessary clearance documentation for our operations. The sooner the JRSO, EPM, and Co-Chief Scientists are aware that microbiology sampling is planned, the better, especially if using contamination tracers. Microbiological research can be limited by several factors, including the expedition's scientific objectives, the implementation of the Nagoya Protocol on Access to Genetic Resources as part of the Convention on Biological Diversity in national waters (Xiao et al., 2018), and the generation of clearance and safety documentation in a timely manner. As of 2020, expeditions in international waters are not constrained by the Nagoya Protocol or other international agreements.

Below, we lay out expectation management for three scenarios on *JOIDES Resolution*: (1) when several microbiologists are sailing

and microbiology-based research is a major objective of the expedition, (2) when two or fewer microbiologists are sailing, and (3) when no microbiologists are sailing but a shore-based participant shows interest and scientists sailing in a different role can assist with shipboard microbiology sampling. Table **T2** summarizes reasonable expectations for each scenario. Our goal is to help scientists new to microbiology sampling on *JOIDES Resolution* prepare as much as possible for what is realistic given the resources and people-power available. Please note that scientists work 12 h shifts every day of the expedition (approximately 60 days) and that the time required for processing different sample lithologies and subsample types (cell count samples, subsamples for DNA extraction, samples for lipid analysis, etc.) will vary. Furthermore, a large component of shipboard work involves the preparation of different reports, which should be comprehensive and therefore require time and effort (we mention this for sailing microbiologists to keep in mind that good time management is essential and not limited to sample processing). Because most IODP microbiology research happens in individual shore-based laboratories, it is especially critical to keep good notes and put as much detail into the reports as possible, both for analyses planned soon after an expedition finishes and for any shore-based participants who plan to use the samples.

What to expect with three or more sailing microbiologists

With three microbiologists on board, it is reasonable to assume the following:

- Microbiological WR samples can be collected and appropriately processed for storage during all shifts (e.g., preserving samples for cell counts, contamination tracer checks, preserving samples in anaerobic bags, freezing interiors for nucleic acid or lipid biomarker extraction). Assume that this is at least one microbiologist's primary role per shift.
- If the expedition plan includes the use of contamination tracers, assume that these can be deployed during all coring operations where microbiological samples will be collected and that the subsamples for quantifying the tracers can be collected during all shifts. Assume that this is at least one microbiologist's secondary role per shift.
- If there is a desire to have the tracer samples analyzed on board for informing sample processing, then assume that this will be one microbiologist's full-time responsibility during their shift because running the gas chromatograph–electron capture detector for this purpose is time-consuming. The chemistry laboratory technician may be able to assist in setting up and monitoring instrument runs, but this assistance is not guaranteed (we recommend discussing this preexpedition with the EPM and then again on board with the EPM, Laboratory Officer, and chemistry technicians). Otherwise, only select samples will be analyzed, and the full sample suite will have to be analyzed on shore using third-party equipment.
- Given the above tasks, the amount of time available for these three microbiologists to do additional sample processing on board the ship will depend on core recovery. If the expedition is sediment coring intensive, there will likely not be much spare time, as core could be coming up relatively rapidly. If the expedition is basement coring intensive, then the amount of time between core recovery is longer. However, in this situation, the amount of time required to process any given core sample is also longer (i.e., cleaning the exterior of the core, breaking the cores open with chisel and hammer, subsampling). Some additional sample analysis and processing may be possible during down times, such as spot cell count measurements, adenosine triphosphate (ATP) quantification, and setting up enrichment experiments or incubations.

With more than three microbiologists on board, it is possible to assume that additional dedicated sample processing for microbiology can occur, such as the following:

- If part of the expedition objectives is to measure microbial activity, bioassays involving radioisotope or stable-isotope incubations will likely be performed as the dedicated role of at least one microbiologist because they are extremely time consuming. These measurement types require the use of the dedicated isotope van on *JOIDES Resolution*, and these needs must be clearly communicated to the JRSO during expedition planning to ensure that they are allowed.
- If part of the expedition objectives includes documenting dissolved oxygen concentrations in sediment cores, then at least one microbiologist would have this task as their sole responsibility during sediment coring operations. It is important to note that (1) as of 2020 this measurement is not standard and requires using third-party equipment and (2) there needs to be a detailed discussion with the science party about modifications to standard core flow to allow oxygen measurements to be taken properly (see the Integrated Ocean Drilling Program Expedition 329 and 336 Methods chapters [Expedition 329 Scientists, 2011; Expedition 336 Scientists, 2012] for examples), such as bringing intact core into *JOIDES Resolution*'s reefer for temperature equilibration and measurement before passing through core scanners and splitting.

What to expect with 1 or 2 sailing microbiologists

- With 1 or 2 dedicated microbiologists on board, their priority must be processing samples for postexpedition analysis. Proper preservation and storage of samples is absolutely crucial. It will not be possible to analyze everything on board, but depending on the sample type and pacing of cores arriving on the catwalk, it may be possible to analyze one or two types of samples, such as cell counts in high-biomass samples (samples requiring density separation for analysis will be too difficult and time-consuming to process with only 1 or 2 dedicated microbiologists), inoculation of enrichment cultures, or ATP quantification.
- Continuous tracer use is possible with 1 or 2 microbiologists on board, but it will need to be balanced with overall expedition scientific objectives and operational strategy at each site(s). Without someone dedicated to tracer testing, it becomes more difficult to ensure that tracers are continuously run and sample tracers from every microbiology WR are collected. It is likely that most if not all of the tracer samples will have to be analyzed postexpedition using third-party equipment.

What to expect with no sailing microbiologists but either a sailing scientist in a different role (i.e., geochemist) or shore-based participants that request sampling during an expedition

- If a microbiologist is sailing in a different capacity (such as a geochemist), they will be expected to complete the tasks necessary for that role and any microbiology sampling or tracer runs will be done as time allows. It is likely that the ability to run tracers

will be very limited and will depend on the expedition's scientific objectives and operational plan. For instance, during a high-recovery expedition there may not be time available to deploy and analyze tracers continually.
- If microbiology samples are requested and if tracers are run, this must be included in all prepared reports and will very likely fall to the scientist(s) moonlighting as a microbiologist. This will be in addition to their writing requirements for their primary job during the expedition. Please keep this in mind if you are considering sailing as something other than a microbiologist but plan to request microbiology samples and/or to conduct tracer runs; it is not impossible to accomplish and is encouraged by the JRSO to maximize the science of any expedition, but it can be very challenging. Sailing scientists are expected to prioritize their shipboard role so that the quality of IODP science is maintained across expeditions.

Regardless of the number of sailing microbiologists, it is important that the science party is aware that sampling for microbiology may need to take precedence to choose WRs that have minimal fracturing/disturbance. This sampling should occur with input from geologists (e.g., geochemists, petrologists, and sedimentologists) to ensure that mission-critical samples for other objectives/specialties are not destroyed. For example, there are times when a core may have only one vein, which is desirable to microbiologists, petrologists, and geochemists. Instances where valuable sediment horizons are sampled (e.g., Paleocene/Eocene Thermal Maximum) are also likely to require compromise with other disciplines.

If you cannot participate as a shipboard scientist but do want to participate as a shore-based scientist, there are considerations that are unique compared to the situations described above. Execution of shore-based sampling requests very much depend on how early a potential shore-based scientist contacts the EPM and Co-Chief Scientists. Assuming that there are no dedicated microbiologists on board, it is possible that only a limited number of samples can be taken, preserved properly, and shipped to you. This is primarily because, as outlined above, without dedicated microbiologists, microbiology sampling would need to be conducted by an onboard scientist during their free time (i.e., taking on an additional role). It is important to also remember that if there is no dedicated microbiologist on board, that the person collecting samples may or may not have experience sampling for microbiology. In this case, it is best to request WR sections or cut-off 10 cm^3 syringe plugs that can be collected and frozen or refrigerated (as needed) immediately after collection with little to no manipulation of the sample required.

For any agreements reached with an expedition's science party regarding sampling for shore-based participants, it is crucial to note that conditions and events at sea can change this plan, sometimes significantly. This is not exclusive to shore-based participants, but preference for material is given to shipboard scientists.

For information on the laboratory spaces, equipment, and instrumentation available aboard *JOIDES Resolution*, please visit **https://wiki.iodp.tamu.edu**.

Conclusions

Microbial contamination tracer use has now allowed several generations of IODP microbiologists to select and analyze quality samples. As the community gained experience in working with these tracers, however, their successes and drawbacks became more apparent. Based on current data and understanding, we suggest that the perfluorocarbon compound PFMD is the best and most reliable tracer because it is less volatile than PMCH and more reliably deployed than fluorescent microspheres. It is not a perfect tracer; scientists must be aware of the caveats and plan sampling and analyses accordingly. Scientists should also make efforts to document tracer use and analytical results so that a greater understanding of best practices can be developed.

This document also outlines strategies for coordinating sampling and analyses among shipboard and shore-based microbiologists. By planning ahead and better understanding what objectives can be reasonably accomplished by varying shipboard microbiology expertise, we can optimize the microbiological research that can be accomplished during an expedition.

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References

Colwell, F.S., Stormberg, G.J., Phelps, T.J., Birnbaum, S.A., McKinley, J., Rawson, S.A., Veverka, C., et al., 1992. Innovative techniques for collection of saturated and unsaturated subsurface basalts and sediments for microbiological characterization. *Journal of Microbiological Methods*, 15(4):279–292. **https://doi.org/10.1016/0167-7012(92)90047-8**

Expedition 329 Scientists, 2011. Methods. *In* D'Hondt, S., Inagaki, F., Alvarez Zarikian, C.A., and the Expedition 329 Scientists (Ed.), *Proceedings of the Integrated Ocean Drilling Program*, 332: Tokyo (Integrated Ocean Drilling Program Management International, Inc.). **https://doi.org/10.2204/iodp.proc.329.102.2011**

Expedition 336 Scientists, 2012. Methods. *In* Edwards, K.J., Bach, W., Klaus, A., and the Expedition 336 Scientists (Ed.), *Proceedings of the Integrated Ocean Drilling Program*, 336: Tokyo (Integrated Ocean Drilling Program International, Inc.). **https://doi.org/10.2204/iodp.proc.336.102.2012**

Fisher, A.T., Tsuji, T., Petronotis, K., and the Expedition 327 Scientists, 2011. *Proceedings of the Integrated Ocean Drilling Program*, 327: Tokyo (IODP Management International). **https://doi.org/10.2204/iodp.proc.327.2011**

Friese, A., Kallmeyer, J., Axel Kitte, J., Montaño Martínez, I., Bijaksana, S., Wagner, D., the ICDP Lake Chalco Drilling Science Team, and the ICDP Towuti Drilling Science Team, 2017. A simple and inexpensive technique for assessing contamination during drilling operations. *Limnology and Oceanography: Methods*, 15(2):200–211. **https://doi.org/10.1002/lom3.10159**

Harvey, R.W., George, L.H., Smith, R.L., and LeBlanc, D.R., 1989. Transport of microspheres and indigenous bacteria through a sandy aquifer: results of natural- and forced-gradient tracer experiments. *Environmental Science & Technology*, 23(1):51–56. **https://doi.org/10.1021/es00178a005**

Hindson, B.J., Ness, K.D., Masquelier, D.A., Belgrader, P., Heredia, N.J., Makarewicz, A.J., Bright, I.J., et al., 2011. High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. *Analytical Chemistry*, 83(22):8604–8610. **https://doi.org/10.1021/ac202028g**

House, C.H., Cragg, B.A., Teske, A., and the Leg 201 Scientific Party, 2003. Drilling contamination tests during ODP Leg 201 using chemical and par-

ticulate tracers. *In* D'Hondt, S.L., Jørgensen, B.B., Miller, D.J. et al. (Ed.), *Proceedings of the Ocean Drilling Program, Initial Reports.* 201: College Station, TX (Ocean Drilling Program), **https://doi.org/10.2973/odp.proc.ir.201.102.2003**

Jutzeler, M., White, J.D.L., Talling, P.J., McCanta, M., Morgan, S., Le Friant, A., and Ishizuka, O., 2014. Coring disturbances in IODP piston cores with implications for offshore record of volcanic events and the Missoula megafloods. *Geochemistry, Geophysics, Geosystems*, 15(9):3572–3590. **https://doi.org/10.1002/2014GC005447**

Kallmeyer, J., 2017. Contamination control for scientific drilling operations. In Sariaslani, S. and Gadd, G.M. (Ed.), Advances in Applied Microbiology (Volume 98): Cambridge, MA (Academic Press), 61–91. **https://doi.org/10.1016/bs.aambs.2016.09.003**

Klein, F., Humphris, S.E., Guo, W., Schubotz, F., Schwarzenbach, E.M., and Orsi, W.D., 2015. Fluid mixing and the deep biosphere of a fossil Lost City-type hydrothermal system at the Iberia Margin. *Proceedings of the National Academy of Sciences of the United States of America*, 112(39):12036–12041. **https://doi.org/10.1073/pnas.1504674112**

Lehman, R.M., Colwell, F.S., Ringelberg, D.B., and White, D.C., 1995. Combined microbial community-level analyses for quality assurance of terrestrial subsurface cores. *Journal of Microbiological Methods*, 22(3):263–281. **https://doi.org/10.1016/0167-7012(95)00012-A**

Lever, M.A., Alperin, M., Engelen, B., Inagaki, F., Nakagawa, S., Steinsbu, B.O., and Teske, A., 2006. Trends in basalt and sediment core contamination during IODP Expedition 301. *Geomicrobiology Journal*, 23(7):517–530. **https://doi.org/10.1080/01490450600897245**

MacLeod, C.J., Dick, H.J.B., Blum, P., Abe, N., Blackman, D.K., Bowles, J.A., Cheadle, M.J., Cho, K., Ciazela, J., Deans, J.R., Edgcomb, V.P., Ferrando, C., France, L., Ghosh, B., Ildefonse, B.M., Kendrick, M.A., Koepke, J.H., Leong, J.A.M., Chuanzhou, L., Qiang, M., Morishita, T., Morris, A., Natland, J.H., Nozaka, T., Pluemper, O., Sanfilippo, A., Sylvan, J.B., Tivey, M.A., Tribuzio, R., and Viegas, L.G.F., 2017. Expedition 360 methods. *In* MacLeod, C.J., Dick, H.J.B., Blum, P., and the Expedition 360 Scientists, *Southwest Indian Ridge Lower Crust and Moho.* Proceedings of the International Ocean Discovery Program, 360: College Station, TX (International Ocean Discovery Program). **https://doi.org/10.14379/iodp.proc.360.102.2017**

McNeill, L.C., Dugan, B., Petronotis, K.E., Backman, J., Bourlange, S., Chemale, F., Jr., Wenhuang, C., Colson, T.A., Frederik, M.C.G., Guèrin, G., Hamahashi, M., Henstock, T., House, B.M., Hüpers, A., Jeppson, T.N., Kachovich, S., Kenigsberg, A.R., Kuranaga, M., Kutterolf, S., Milliken, K.L., Mitchison, F.L., Mukoyoshi, H., Nair, N., Owari, S., Pickering, K.T., Pouderoux, H.F.A., Yehua, S., Song, I., Torres, M.E., Vannucchi, P., Vrolijk, P.J., Tao, Y., and Zhao, X., 2017. Expedition 362 methods. *In* McNeill, L.C., Dugan, B., Petronotis, K.E., and the Expedition 362 Scientists, *Sumatra Subduction Zone.* Proceedings of the International Ocean Discovery Program, 362: College Station, TX (International Ocean Discovery Program). **https://doi.org/10.14379/iodp.proc.362.102.2017**

McNeill, L.C., Dugan, B., Petronotis, K.E., Backman, J., Bourlange, S., Chemale, F., Jr., Wenhuang, C., Colson, T.A., Frederik, M.C.G., Guèrin, G., Hamahashi, M., Henstock, T., House, B.M., Hüpers, A., Jeppson, T.N., Kachovich, S., Kenigsberg, A.R., Kuranaga, M., Kutterolf, S., Milliken, K.L., Mitchison, F.L., Mukoyoshi, H., Nair, N., Owari, S., Pickering, K.T., Pouderoux, H.F.A., Yehua, S., Song, I., Torres, M.E., Vannucchi, P., Vrolijk, P.J., Tao, Y., and Zhao, X., 2020. IODP Expedition 362 closeup images [data set]. Zenodo. **https://doi.org/10.5281/zenodo.3751835**

Mills, H.J., Reese, B.K., and St. Peter, C., 2012. Characterization of microbial population shifts during sample storage. *Frontiers in Microbiology*, 3:49. **https://doi.org/10.3389/fmicb.2012.00049**

Orcutt, B.N., Bergenthal, M., Freudenthal, T., Smith, D., Lilley, M.D., Schnieders, L., Green, S., and Früh-Green, G.L., 2017. Contamination tracer testing with seabed drills: IODP Expedition 357. *Scientific Drilling*, 23:39–46. **https://doi.org/10.5194/sd-23-39-2017**

Orcutt, B.N., LaRowe, D.E., Lloyd, K.G., Mills, H., Orsi, W., Reese, B.K., Sauvage, J., Huber, J.A., and Amend, J., 2014. IODP Deep Biosphere Research Workshop report – a synthesis of recent investigations, and discussion of new research questions and drilling targets. *Scientific Drilling*, 17:61–66. **https://doi.org/10.5194/sd-17-61-2014**

Pearson, P.N., and Nicholas, C.J., 2014. Layering in the Paleocene/Eocene boundary of the Millville core is drilling disturbance. *Proceedings of the National Academy of Sciences of the United States of America*, 111(12):E1064–E1065. **https://doi.org/10.1073/pnas.1322077111**

Sauvage, J., Lewis, L., Graham, D., Spivack, A.J., and D'Hondt, S., 2017. Data report: quantification of potential drilling contamination using perfluorocarbon tracer at IODP Expedition 329 sites. *In* D'Hondt, S., Inagaki, F., Alvarez Zarikian, C.A., and the Expedition 329 Scientists (Ed.), *Proceedings of the Integrated Ocean Drilling Program.* 329: Tokyo (Integrated Ocean Drilling Program Management International, Inc.), **https://doi.org/10.2204/iodp.proc.329.204.2017**

Sheik, C.S., Reese, B.K., Twing, K.I., Sylvan, J.B., Grim, S.L., Schrenk, M.O., Sogin, M.L., and Colwell, F.S., 2018. Identification and removal of contaminant sequences from ribosomal gene databases: lessons from the census of deep life. *Frontiers in Microbiology*, 9:840. **https://doi.org/10.3389/fmicb.2018.00840**

Smith, D.C., Spivack, A.J., Fisk, M.R., Haveman, S.A., Staudigel, H., and the Leg 185 Shipboard Scientific Party, 2000. Methods for quantifying potential microbial contamination during deep ocean coring. *Ocean Drilling Program Technical Note*, 28. **https://doi.org/10.2973/odp.tn.28.2000**

Xiao, N., Ahagon, N., Kubo, Y., and Morioka, H., 2018. Preparing for the new age of the Nagoya Protocol in scientific ocean drilling. *Scientific Drilling*, 24:51–59. **https://doi.org/10.5194/sd-24-51-2018**

Yanagawa, K., Nunoura, T., McAllister, S.M., Hirai, M., Breuker, A., Brandt, L., House, C.H., et al., 2013. The first microbiological contamination assessment by deep-sea drilling and coring by the D/V *Chikyu* at the Iheya North hydrothermal field in the mid-Okinawa Trough (IODP Expedition 331). *Frontiers in Microbiology*, 4:327. **https://doi.org/10.3389/fmicb.2013.00327**

Table T1. Microbiology (MBIO) sample repositories maintained in the three IODP core repositories.

Location of MBIO repository	Name of MBIO repository	Website
Gulf Coast Repository	Gulf Coast Repository MBIO	http://iodp.tamu.edu/curation/imagerep/microbiology
Kochi Core Repository	DeepBIOS	http://www.kochi-core.jp/DeepBIOS/
Bremen Core Repository	Bremen Core Repository	https://www.marum.de/en/Research/IODP-Bremen-Core-Repository.html#Section1639

Table T2. Planning table to guide sampling, tracer use, and experiments attempted shipboard as a function of sailing microbiologists. Est. = estimated. FTE = full-time employee. PFT = perfluorocarbon tracer.

Role of microbiology in expedition planning		**Expectations for microbiology-related shipboard science, QA/QC, sample collection, and storage protocols**		
Research questions or science relevance	Number of shipboard microbiologists	Est. total microbiology samples collected during expedition	Est. shipboard FTE to sample and conduct QA/QC	Recommended shipboard methods for sample QA/QC, collection, preservation, and analysis
None apparent	0	0	0	—
Some relevance (e.g., ≥1 shore-based requests)	1	1–20	0.5–1	Drilling fluid, seawater routinely collected for comparison (~10 samples); whole rounds collected and frozen intact for postexpedition analysis; minimal/no shipboard experiments. Collection of samples for 1 or 2 shore-based scientists possible.
Part of expedition science objectives	2–3	20–100	2	Modest to full contamination tracer program. Samples collected for shipboard and shore-based scientists. Shipboard experiments; some real-time analyses that may inform drilling targets.
As central theme for expedition objectives	3+	>100	>2	Full contamination tracer program (PFTs) adapted to samples expected. Sample collection and preservation for studies. New methods, materials explored, different geological media examined. Consider development, testing of new tracer strategies. Shipboard studies and experiments influence coring targets.

Figure F1. Coring systems used on *JOIDES Resolution*. I.D. = inner diameter, O.D. = outer diameter.

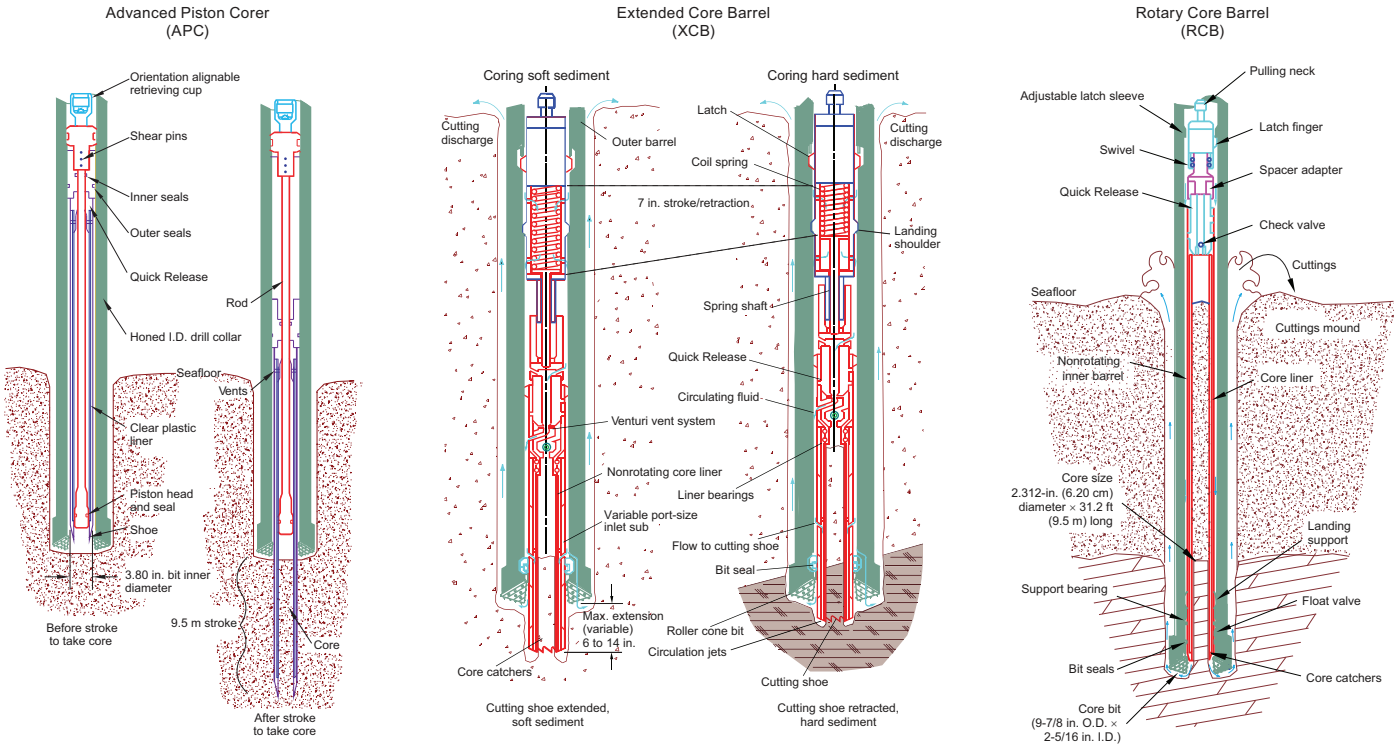

Figure F2. A–C. Examples of sediment core disturbance: (A) fall-in sampled during IODP Expedition 362 (McNeill et al., 2017), (B) uparching sediment (McNeill et al., 2017), (C) "biscuits and gravy" generated during RCB coring (362-U1480G-8R; McNeill et al., 2020). Scales are in centimeters. D. Example of basement core pieces showing piece taken from Section 327-U1362A- 6R-1 for microbiological sampling (where ruler starts at 0) versus smaller "roller" pieces further down in the core. Original photo courtesy of William Crawford/IODP JRSO and available in MICROBIO in the Integrated Ocean Drilling Program Expedition 327 Supplementary material (Fisher, Tsuji, Petronotis, and the Expedition 372 Scientists, 2011).

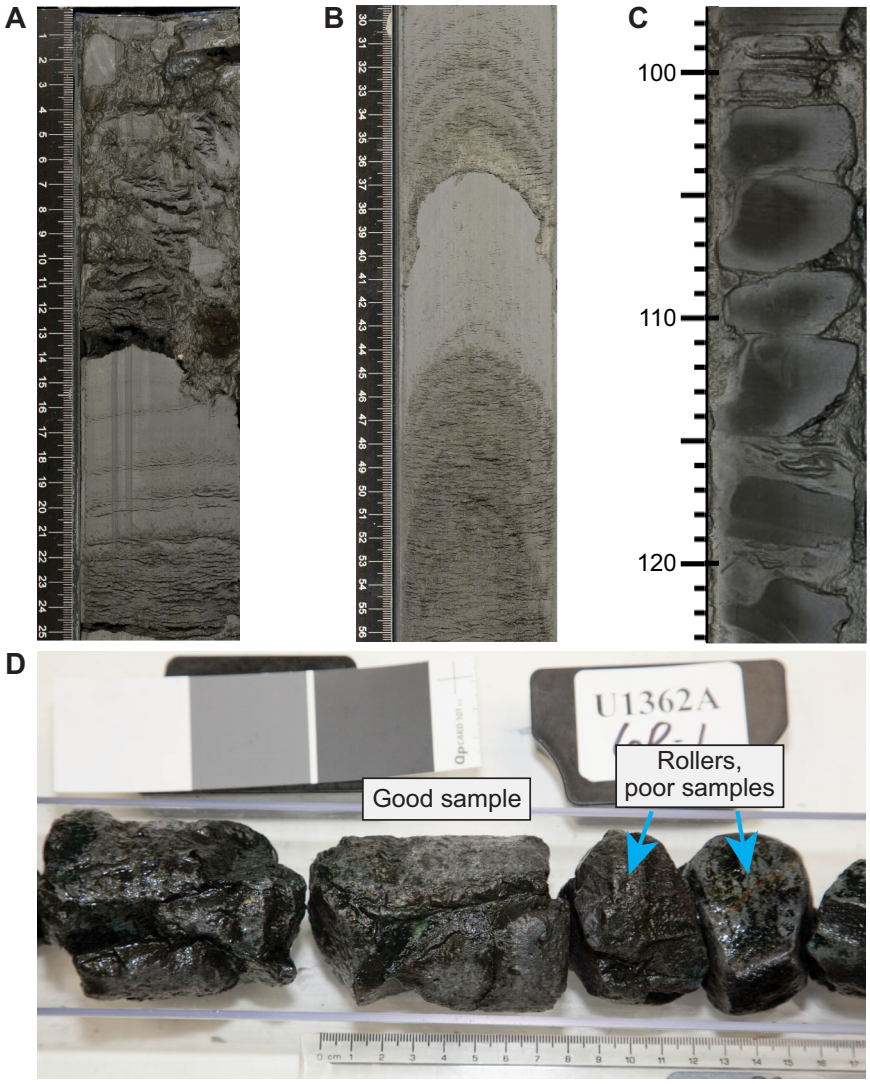

Figure F3. Examples of (A, B) positive pressure sampling area with a HEPA filter to clean incoming air and KOACH bench (C) setup and (D) in use during Expedition 360 (MacLeod et al., 2017). Both setups create a clean environment for microbiological sampling. The KOACH bench can be set up in an anaerobic chamber and/or in the cold room. Photos courtesy of Yuki Morono.

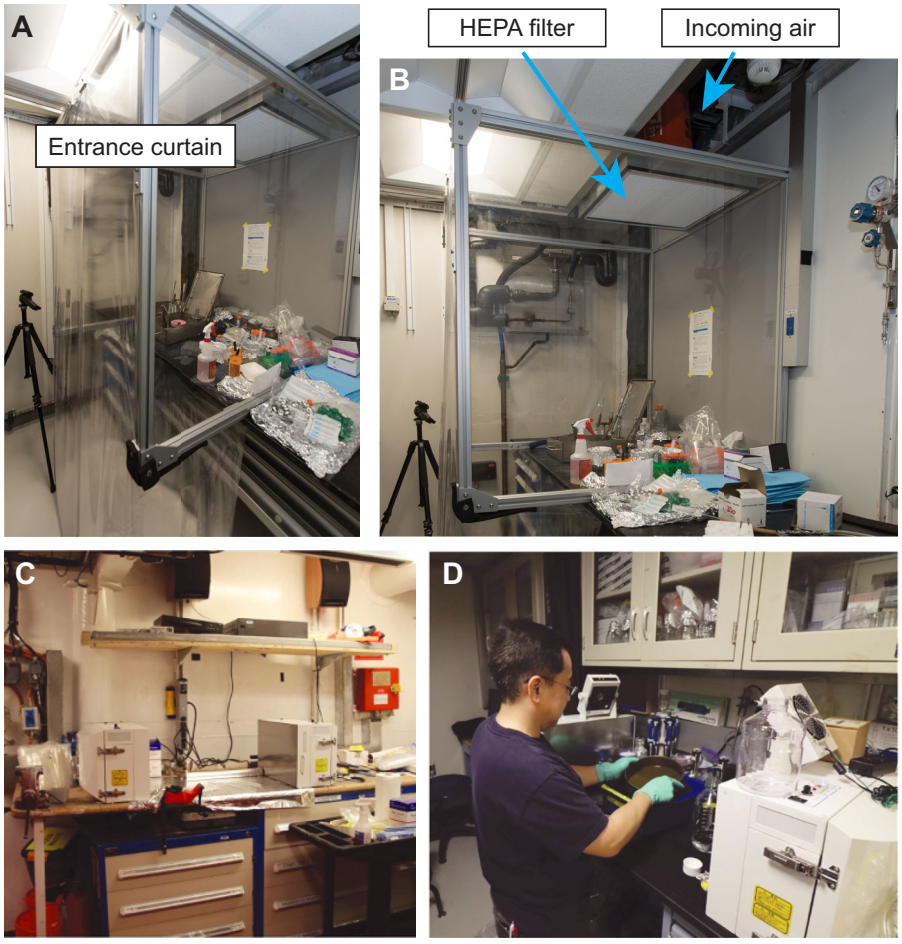

Figure F4. Demonstration of microsphere deployment: (A, B) Microspheres in Whirl-Pak bag draped over core catcher, (C) top-down view, (D) bottom-up view showing how core will rip through Whirl-Pak bag and release microspheres when deployment succeeds, (E) instance of a failed deployment where Whirl-Pak bag is lodged in the core without even distribution of microspheres (Integrated Ocean Drilling Program Expedition 330; photo by J. Sylvan).