FISH TISSUE PFAS SAMPLING

Guidance

Introduction

This sampling guidance discusses processes, decontamination procedures, and acceptable materials for sampling of fish for per- and polyfluoroalkyl substances (PFAS). The guidance will supplement the **MDEQ-WRD Fish Contaminant Monitoring Program (FCMP) Fish Collection Procedure (WRD-SWAS-004)** but does not replace it. In addition, this sampling guidance will be used to support the sampling objectives and procedures based on the Quality Assurance Project Plan (QAPP) developed prior to any field activities. This guidance assumes staff has basic familiarity with and/or understanding of basic fish sampling procedures.

NOTE: Review the **General PFAS Sampling Guidance** prior to reviewing this guidance document.

The Michigan Department of Environmental Quality (MDEQ) intends to update the information contained within this PFAS Sampling Guidance document as new information becomes available. The user of this PFAS Sampling Guidance is encouraged to visit the Michigan PFAS Action Response Team webpage (www.michigan.gov/PFASresponse) to access the current version of this document.

Some PFAS, such as perfluorooctanesulfonic acid (PFOS), are known to bioaccumulate in fish. Limited PFAS biota samples have been collected in Michigan to date, with the highest detection concentration of over 9,000 parts per billion (ppb) in fish filet and over 70,000 ppb in fish liver. Michigan Fish Consumption Screening Values (FCSV) for PFOS range between 9 ppb (16 servings per month category) up to 300 ppb (Do Not Eat).

NOTE: Fish tissue analysis for PFAS produces results in ppb while ambient water analysis measures PFAS in parts per trillion. Concentrations of PFOS in particular are several orders of magnitude higher in fish than in the ambient surface water where the fish were collected. Even so, PFOS was below the detection limit of 0.25 ppb in 4% of the 760 individual fish fillet samples analyzed as of 2017. In addition, as of 2017, perfluorooctanoic acid (PFOA) was below the detection limit in over 90% of the samples, and on average the other 9 PFAS analyzed regularly for the MDEQ Fish Contaminant Monitoring Program were below detection in 67% of the samples.

To date, it has not been documented that cross-contamination of fish during collection and sample processing outside of the analytical laboratory setting will result in a significant impact on the concentrations measured in tissue samples, but staff must be aware of potential PFAS sources and take precautions to minimize possible effects on the analytical results.

This fish tissue sampling guidance discusses the collection of fish samples for PFAS and methods to prevent cross-contamination that can occur from:

- Field clothing and personal protective equipment (PPE)
- Personal care products (PCPs)
- Food Packaging
- Sampling equipment
- Sample collection and handling
- Sample shipment

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NOTE: Additional information about PFAS testing can be found on the Michigan PFAS Action Response Team (MPART) website: www.michigan.gov/PFASresponse

● – Prohibited ■ – Allowable ▲ – Needs Screening

1. Fish Selection

Several fish species can be collected for PFAS and the species chosen depends on the target water body and project objectives. Most bioaccumulative contaminants tend to increase in concentration in tissues of organisms at successively higher levels in a food chain, a process known as biomagnification. For example, mercury concentrations in top predator fish (e.g., walleye, largemouth bass) can be orders of magnitude higher than in their prey. However, biomagnification does not seem to be a significant factor with PFOS. Sampling in Michigan and elsewhere has shown that top predator fish can have similar or lower concentrations of PFOS compared to lower trophic level fish from the same water body.

Several factors should be considered when selecting the fish species that will be targeted for collection:

- Fish species and sizes normally consumed by anglers should be selected if results are expected to be used by the Michigan Department of Health and Human Services (MDHHS) “Eat Safe Fish” Program.
- If ecological risk assessment is a project goal, then fish of a size and species likely to be eaten by piscivorous birds and mammals should be targeted.
- Consider targeting species to match samples from other Michigan waters that have been analyzed for PFAS. This allows spatial comparisons without the potential complications of inter species differences.
- Migratory patterns of a species should be considered when sampling in a river reach open to the Great Lakes if the results are to be used to evaluate water quality in the stream. A species that is highly mobile or exhibits seasonal migrations may not be sufficiently representative of conditions in the stream of capture.

The ideal sample size consists of a total of at least 10 fish and includes both sexes. Although several studies have indicated there is not a strong correlation between PFAS concentrations and fish length, an ideal sample will include fish in a range of lengths because other contaminants will be assayed in the samples and concentrations for most contaminants do tend to increase with fish size. The MDHHS protocol for determining the need for fish consumption advisories requires a minimum sample size of five fish of legal size.

Because most fish tissue samples analyzed for the MDEQ FCMP are used to develop consumption advisories for the MDHHS “Eat Safe Fish” Program, they should represent fish likely to be eaten by anglers. Therefore, fish with abnormal deformities, gross internal lesions, or other obvious health issues should not be sampled.

2. Potential Sources for PFAS Cross-Contamination

Potential sources for PFAS cross-contamination are materials used within the sampling environment, sampling equipment, field clothing, personal protective equipment (PPE), sun and biological protection products, personal hygiene, and personal care products (PCPs), food packaging, and the environment itself. A detailed discussion about potential sources for PFAS cross-contamination is discussed in the **General PFAS Sampling Guidance**, which should be reviewed before reading this document. However, a high-level summary will be presented in this guidance.

All of the materials or items discussed in the Fish Tissue PFAS Sampling Guidance will be divided into three major groups:

- Prohibited (●) identifies items and materials that should not be used when sampling. It is well documented that they contain PFAS or that PFAS are used in their manufacture.

- Allowable (■) identifies items and materials that have been proven not to be sources of PFAS cross contamination and are considered acceptable for sampling.

- Needs Screening (▲) identifies items and materials that have the potential for PFAS cross-contamination due to a lack of scientific data or statements from manufacturers to prove otherwise. These items and materials are further sub-divided into two categories:

    - **Category 1:** Items and materials that will come in direct contact with the sample. These should not be used when sampling unless they are known to be PFAS-free, by collecting an equipment blank sample prior to use.

    - **Category 2:** Items and materials that will not come in direct contact with the sample. These should be avoided, if possible, unless they are known to be PFAS-free by collecting an equipment blank sample prior to use.

Please note that at this time no published research is available that documents the use of various materials and effect on sample results. Therefore, a conservative approach is recommended, and the guidance is based on the collection of multiple environmental samples at various PFAS Sites. Sampling staff should take practical and appropriate precautions to avoid items that are likely to contain PFAS at the sampling site as well as avoid specific items during the sampling event.

A general overview of PFAS contamination sources during sampling can be found in **Section 4.2** of the **General PFAS Sampling Guidance**. Any items or materials utilized that are not identified in this guidance or not discussed in **Section 4.2** should be evaluated as described in **Section 4.2.1**.

Sampling staff should take practical and appropriate precautions to avoid items that are likely to contain PFAS at the sampling site as well as avoid specific items during the sampling event (see below).

2.1 Field Clothing and Personal Protection Equipment (PPE)

A general overview of field clothing and PPE can be found in **Section 4** of the **General PFAS Sampling Guidance**.

While preparing for fishing and sampling fish, be particularly cautious of clothing that has been advertised as having waterproof, water-repellant, or stain-resistant characteristics, as these properties contain PFAS. Prior to using the item in question, consult with a PFAS technical expert if there are any questions about whether a material contains PFAS.

NOTE: Ensuring that all the field clothing and PPE are made of PFAS-free materials is considered precautionary. However, these items are not expected to come into contact with the actual fish samples.

Life jackets must be used during fish collection and should be made of PFAS-free materials if available. Waders should be made of PFAS-free materials, if possible. Elimination of certain

NOTE: Protective coatings that could contain PFAS might still be used in the manufacturing of life jackets.

materials, clothing, or fishing equipment during fish sampling, however, may not be possible. **The safety of the field staff must not be compromised.**

As with any field mobilization, it is the responsibility of all personnel to be aware of the physical, chemical and biological hazards associated with a particular site. Personal safety is paramount. The safety of staff should not be compromised by fear of PFAS-containing materials without any scientific basis. Any deviation from this guidance, including those necessary to ensure the health and safety of sampling personnel, should be recorded in field notes and discussed in the final report.

Clothing materials that should be avoided if possible, include the following:

- Any known fluoropolymers that contain PFAS such as, but not limited to, Polytetrafluoroethylene (PTFE known as Teflon®), Polyvinylidene fluoride (PVDF), Polyvinyl fluoride (PVF), and Fluorinated ethylene propylene (FEP), or Perfluoropolyethers (PFPE).
- Clothing or boots containing Gore-Tex®.
- Clothing washed with fabric softener may contain PFAS.
- Chemically treated clothing for insect resistance and ultraviolet (UV) protection.

Clothing materials that are acceptable include the following:

- Life jackets made of polyethylene foam and nylon shell fabric can be used.
- Waders made of polyvinyl chloride (PVC) or neoprene can be used.
- PVC or wax-coated fabrics.
- Neoprene.
- Synthetic and natural fibers (preferably cotton).
- Any boots made of polyurethane and PVC.
- Clothes that have been well laundered several times and without fabric softener from time of purchase.

Note: Manufacturers can change the chemical composition of any product. As a result, all materials that will come into direct contact with the sample media should be tested to confirm they are "PFAS-free," i.e. will not contaminate samples at detectable levels. **There is no guarantee that materials in the 'Acceptable' category will always be PFAS- free.**

Any field clothing and/or PPE items that might be required for surface water sampling and not discussed in this guidance should be evaluated as described in **Section 4.2.2** of the **General PFAS Sampling Guidance**.

Powderless nitrile gloves should frequently be changed any time there is an opportunity for cross-contamination of the sampling including, but not limited to, the following activities:

- Each time sampling equipment is handled.
- Prior to sample collection.
- After handling any sample, including QA/QC samples such as field reagent blanks or equipment rinsate blanks.
- After the handling of any non-dedicated sampling equipment, contact with non-decontaminated surfaces, or when judged necessary by field personnel.
- During and after decontamination of non-dedicated sampling equipment.

2.2 Field Personal Care Products (PCPs)

A number of sampling guidance documents recommend that personal hygiene and personal care products (PCPs) (e.g., cosmetics, shampoo, sunscreens, dental floss, etc.) not be used prior to and on the day(s) of sampling because the presence of PFAS in these products has been documented

(OECD, 2002, Fujii, 2013, Borg and Ivarsson, 2017). However, if the MDEQ's sampling standard operating procedures are followed, these items should not come into contact with the sampling equipment or the sample being collected. As of the date of this sampling guidance, cross-contamination of samples due to the use of PCPs has not been documented during the collection of thousands of samples. However, field personnel should be aware of the potential of cross-contamination if the sampling equipment or actual samples would come into contact with these products.

- ● Do not handle or apply PCPs in the sampling area.
- ● Do not handle or apply PCPs while wearing PPE that will be present during sampling.
- ■ Move to the staging area and remove PPE if applying personal care products becomes necessary.
- ■ Wash hands thoroughly after the handling or application of PCPs and, when finished, put on a fresh pair of powderless nitrile gloves.

2.3 Food Packaging

PFAS has been used by the paper industry as a special protective coating against grease, oil, and water for paper and paperboards, including food packaging since the late 1950s (Trier et al., 2018). PFAS application for food packaging includes paper products that come into contact with food such as paper plates, food containers, bags, and wraps (OECD, 2002). Pre-wrapped food or snacks (such as candy bars, microwave popcorn, etc.) must not be in the sampling and staging areas during sampling due to PFAS contamination of the packaging. When staff requires a break to eat or drink, they should remove their gloves, coveralls, and any other PPE, if worn, in the staging area and move to the designated area for food and beverage consumption. When finished, staff should wash their hands and put on a fresh pair of powderless nitrile gloves at the staging area, before returning to the sampling area.

- ● Do not handle, consume, or otherwise interact with pre-wrapped food or snacks, carry-out food, fast food, or other food items while on-site during sampling.
- ■ Move to the staging area and remove PPE prior to leaving the sampling and staging areas if consuming food on site becomes necessary.

3. Fish Collection

A wide variety of gear can be used to collect fish. The methods used depend on the species of fish targeted, water body type to be sampled, and the time of year. Regardless of the collection method used, most of the sampling equipment can be considered **Category 2**, which, as defined in the General PFAS Sampling Guidance, are field equipment that does not come into contact with the fish. Examples of **Category 2** equipment include boats, electrofishing equipment, GPS receivers, notebooks, and other sampling equipment used to collect or document the sampling. Approved powderless nitrile gloves should be used whenever any **Category 2** sampling equipment will be used. Frequently changing powderless nitrile gloves should be protective enough to avoid any cross contamination from **Category 2** sampling equipment items in case any of them might contain PFAS.

Fish collected for the MDEQ FCMP are rinsed with ambient water in the field to remove any vegetation or debris, placed in polyethylene bags and on ice in coolers, and frozen as soon as is practical. Fish are then delivered to the MDEQ warehouse in Lansing where they are held frozen until being processed. Fish are thawed in the original bags overnight prior to processing.

3.1 Fish Tissue Sampling Equipment

General sampling equipment that will come into contact with the fish tissue sample (defined in **Section 4.2** of the General PFAS Sampling Guidance as **Category 1**) should be PFAS free. All tissue samples will come into contact with knives, wrapping material, and sample bags, regardless of sample type. In addition, samples will be in contact with a measuring board, weight scale, and the processing table. The MDEQ-FCMP uses stainless-steel processing tables and knives, wooden (untreated) measuring boards, and scales with a stainless-steel hook for fish attachment. Measuring boards, knives, and tables are rinsed with municipal water before processing begins and between each fish sample.

NOTE: As a precautionary action collection of an equipment rinsate blank could be considered even if the sampling materials are made of materials that are not expected to contain PFAS.

3.2 Sample Processing and Handling

Fish tissue samples that will be used for the MDHHS "Eat Safe Fish" Program are prepared as "standard edible portions." The type of sample is species-dependent and either skin-off fillet, skin-on fillet, boneless steak, or gutted/headless **(WRD-SWAS-004)**. Samples to be analyzed for the MDEQ FCMP temporal trend sampling element or as part of caged fish studies are analyzed as whole fish or whole fish composites, respectively.

Project-specific objectives such as the complete list of biota tissues (e.g., fillets and various organs) should be defined and established before sample collection. Any sampling containers should be able to withstand temperatures as low as -20°C. A list of unacceptable and acceptable sampling containers can be found below.

- ● Sampling containers, bags, or cutting surfaces that are known to use PFAS during manufacturing or are fluoropolymers such as, but not limited to, PTFE (known as Teflon®), PVDF, PVF, or FEP should not be used during sampling for any purposes, and especially during tissue collection.
- ● Do not use clipboards made of plastic.
- ■ Polyethylene plastic freezer bags (e.g., Ziploc® bags) and/or aluminum foil should be used to store any biota tissue samples (existing sample processing and information from the Minnesota Department of Health and Ontario Ministry of Environment and Climate Change have indicated that using aluminum foil [dull side toward the sample] is not a source of measurable cross-contamination).
- ■ Powderless nitrile gloves should be used when handling fish samples.
- ■ A fine point Sharpie can be used to label wrapped samples and sample bags. Preprinted labels can also be used.
- ■ Notes should be taken on loose paper that is kept in aluminum or Masonite® clipboards.

The tissue sampling should occur in the following sequence:

1) Hands must be washed before beginning the sampling event, and clean, powderless nitrile gloves must be worn before handling sample bags and equipment.
2) Fish collected for the MDEQ FCMP are held frozen until being processed, then thawed overnight in the original collection bags prior to processing.
3) Fish should be rinsed thoroughly before tissue sample collection.
4) The tissue collection (fillets and/or organs as specified in the project specific workplan or QAPP) should be collected from healthy, uninjured fish.

●– Prohibited ■– Allowable ▲– Needs Screening

5) Tissue samples are processed, wrapped, and labeled following the **MDEQ Fish Collection Procedure (WRD-SWAS-004)**.
6) Tissue samples should be placed in the MDEQ freezer immediately and kept frozen until delivery to the laboratory for analysis.
7) Knives and surface areas used to process tissue samples must be rinsed between tissue samples.

3.3 Periodic Quality Control Testing

Analysis of PFAS concentrations in fish tissue by other agencies (Minnesota Department of Health; Ontario Ministry of Environment and Climate Change) indicates that cross contamination during the collection and processing of samples prior to delivery to the analytical laboratory is unlikely to affect the results. Normal fish tissue processing procedures can be followed as long as staff are aware of likely sources of PFAS contamination and take reasonable precautions to avoid cross contamination while collecting, measuring, and filleting fish.

Periodic checks of the fish filleting process done by senior staff should be conducted to determine if methods or equipment need to be modified.

4. Sample Shipment

The MDHHS requires that fish used for the “Eat Safe Fish” consumption advisory program are analyzed by their Chemistry and Toxicology Division, Analytical Chemistry Laboratory in Lansing, Michigan. All fish tissue samples for the MDEQ FCMP are analyzed for PFAS and other contaminants by that laboratory.

The MDEQ FCMP samples are kept frozen and delivered directly to the laboratory by MDEQ staff when requested by MDHHS laboratory staff. Sample shipment to other analytical laboratories would require other arrangements and protocols as determined by the receiving laboratory.

Michigan Department of Environmental Quality

MDEQ PFAS Sampling Quick Reference Field Guide[1]

All Items Used During Sampling Event

● Prohibited

• Items or materials that contain fluoropolymers such as
  ○ Polytetrafluoroethylene (PTFE), that includes the trademarks Teflon® and Hostaflon®
  ○ Polyvinylidene fluoride (PVDF), that includes the trademark Kynar®
  ○ Polycholotrifluoroethylene (PCTFE), that includes the trademark Neoflon ®
  ○ Ethylene-tetrafluoro-ethylene (ETFE), that includes the trademark Tefzel®
  ○ Fluorinated ethylene propylene (FEP), that includes the trademarks Teflon® FEP and Hostaflon® FEP
• Items or materials that contain any other fluoropolymer

Pumps, Tubing, and Sampling Equipment

● Prohibited	■ Allowable	▲Needs Screening[2]

• Items or materials containing any fluoropolymer (potential items include tubing, valves, or pipe thread seal tape)	• High-density polyethylene (HDPE)
	• Low-density polyethylene (LDPE) tubing
	• Polypropylene
	• Silicone
	• Stainless-steel
	• Any items used to secure sampling bottles made from:
	  ○ Natural rubber
	  ○ Nylon (cable ties)
	  ○ Uncoated metal springs
	  ○ Polyethylene
		• Any items or materials that will come into direct contact with the sample that have **not** been verified to be PFAS-free
		  ○ Do not assume that any sampling items or materials are PFAS-free based on composition alone

Sample Storage and Preservation

● Prohibited	■ Allowable	▲Needs Screening[2]
• Polytetrafluoroethylene (PTFE): Teflon® lined bottles or caps	• Glass jars[4] • Laboratory-provided PFAS-Free bottles: ○ HDPE or polypropylene • Regular wet ice • Thin HDPE sheeting • LDPE resealable storage bags (i.e. Ziploc®) that will not contact the sample media[6]	• Aluminium foil[4] • Chemical or blue ice[5] • Plastic storage bags other than those listed as ■ Allowable • Low-density polyethylene (LDPE) bottles

Field Documentation

● Prohibited	■ Allowable	▲Needs Screening[2]
• Clipboards coated with PFAS • Notebooks made with PFAS treated paper • PFAS treated loose paper • PFAS treated adhesive paper products	• Loose paper (non-waterproof, non-recycled) • Rite in the Rain® notebooks • Aluminium, polypropylene, or Masonite field clipboards • Ballpoint pens, pencils, and Fine or Ultra-Fine Point Sharpie® markers	• Plastic clipboards, binders, or spiral hard cover notebooks • All markers not listed as ■ Allowable • Post-It® Notes or other adhesive paper products • Waterproof field books

Decontamination

● Prohibited	■ Allowable	▲Needs Screening[2]
• Decon 90® • PFAS treated paper towel	• Alconox®, Liquinox®, or Citranox® • Triple rinse with PFAS-free deionized water • Cotton cloth or untreated paper towel	• Municipal water • Recycled paper towels or chemically treated paper towels

Clothing, Boots, Rain Gear, and PPE

● Prohibited	■ Allowable	▲Needs Screening[2]

• New or unwashed clothing
• Anything made of or with:
  ○ Gore-Tex™ or other water-resistant synthetics
• Anything applied with or recently washed with:
  ○ Fabric softeners
  ○ Fabric protectors, including UV protection
  ○ Insect resistant chemicals
  ○ Water, dirt, and/or stain resistant chemicals
	• Powderless nitrile gloves
	• Well-laundered synthetic or 100% cotton clothing, with most recent launderings not using fabric softeners
	• Made of or with:
	  ○ Polyurethane
	  ○ Polyvinyl chloride (PVC)
	  ○ Wax coated fabrics
	  ○ Rubber / Neoprene
	  ○ Uncoated Tyvek®
		• Latex gloves
		• Water and/or dirt resistant leather gloves
		• Any special gloves required by a HASP
		• Tyvek® suits, clothing that contains Tyvek®, or coated Tyvek®

Food and Beverages

● Prohibited	■ Allowable

• No food should be consumed in the staging or sampling areas, including pre-packaged food or snacks.
  ■ If consuming food on-site becomes necessary, move to the staging area and remove PPE. After eating, wash hands thoroughly and put on new PPE.
	• Brought and consumed only outside the vicinity of the sampling area:
	  ○ Bottled water
	  ○ Hydration drinks (i.e. Gatorade®, Powerade®)

Personal Care Products (PCPs) - for day of sample collection[6]

● Prohibited	■ Allowable	▲Needs Screening[2]
• Any PCPs[6], sunscreen, and insect repellent applied in the sampling area.	PCPs[6], sunscreens, and insect repellents applied in the staging area, away from sampling bottles and equipment followed by thoroughly washing hands: **PCPs[6]:** • Cosmetics, deodorants/antiperspirants, moisturizers, hand creams, and other PCPs[6] **Sunscreens:** • Banana Boat® for Men Triple Defense Continuous Spray Sunscreen SPF 30 • Banana Boat® Sport Performance Coolzone Broad Spectrum SPF 30 • Banana Boat® Sport Performance Sunscreen Lotion Broad Spectrum SPF 30 • Banana Boat® Sport Performance Sunscreen Stick SPF 50 • Coppertone® Sunscreen Lotion Ultra Guard Broad Spectrum SPF 50 • Coppertone® Sport High Performance AccuSpray Sunscreen SPF 30 • Coppertone® Sunscreen Stick Kids SPF 55 • L'Oréal® Silky Sheer Face Lotion 50 • Meijer® Clear Zinc Sunscreen Lotion Broad Spectrum SPF 50 • Meijer® Sunscreen Continuous Spray Broad Spectrum SPF 30 • Meijer® Clear Zinc Sunscreen Lotion Broad Spectrum SPF 15, 30 and 50 • Meijer® Wet Skin Kids Sunscreen Continuous Spray Broad Spectrum SPF 70 • Neutrogena® Beach Defense Water+Sun Barrier Lotion SPF 70 • Neutrogena® Beach Defense Water+Sun Barrier Spray Broad Spectrum SPF 30 • Neutrogena® Pure & Free Baby Sunscreen Broad Spectrum SPF 60+ • Neutrogena® UltraSheer Dry-Touch Sunscreen Broad Spectrum SPF 30 **Insect Repellents:** • OFF® Deep Woods • Sawyer® Permethrin	• Products other than those listed as ■Allowable

[1] This table is not considered to be a complete listing of prohibited or allowable materials. All materials should be evaluated prior to use during sampling. The manufacturers of various products should be contacted in order to determine if PFAS was used in the production of any particular product.

[2] Equipment blank samples should be taken to verify these products are PFAS-free prior to use during sampling.

[3] **For surface water foam samples:** LDPE storage bags may be used in the sampling of foam on surface waters. In this instance, it is allowable for the LDPE bag to come into direct contact with the sample media.

[4] **For fish and other wildlife samples:** Depending on the project objectives, glass jars and aluminum foil might be used for PFAS sampling. PFAS has been found to bind to glass and if the sample is stored in a glass jar, a rinse of the jar is required during the sample analysis. PFAS are sometimes used as a protective layer for some aluminum foils. An equipment blank sample should be collected prior to any aluminum foil use.

[5] Regular ice is recommended as there are concerns that chemical and blue ice may not cool and maintain the sample at or below 42.8°F (6°C) (as determined by EPA 40 CFR 136 – NPDES) during collection and through transit to the laboratory.

[6] Based on evidence, avoidance of PCPs is considered to be precautionary because none have been documented as having cross-contaminated samples due to their use. However, if used, application of PCPs must be done at the staging area and away from sampling bottles and equipment, and hands must be thoroughly washed after the use of any PCPs prior to sampling.

● - Prohibited ■ - Allowable ▲ - Needs Screening