Methods for the Collection of Selenium Residue in Fish Tissue Used to Determine KPDES Permit Compliance

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12/9/19	All	Document was thoroughly revised with addition of additional water body types, target species, sample type and reference to homogenization SOP.

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Scope and Applicability

This standard operating procedure (SOP) has been developed by the Division of Water for the uniform collection of selenium residue in fish tissue for the purposes of compliance with KPDES permits. The methods set forth herein are required for all activities related to the collection of fish for the determination of selenium residue in fish tissue.

Definitions and Acronyms

Division: Kentucky Division of Water

KPDES: Kentucky Pollutant Discharge Elimination System

Large Streams: Free-flowing streams with a catchment area of 20-200 mi^2. Most of the sampling reach is wadeable with a mean average thalweg depth less than 1 meter. Small areas may be non-wadeable.

Littoral Zone: Shallow shore areas where light can penetrate to the bottom.

Non-wadeable: Stream sections that cannot be traversed by foot and sampling cannot be performed without the aid of a boat.

Predator: Includes black bass (smallmouth, largemouth and spotted), white bass, striped bass, sauger, saugeye, walleye, muskellunge, flathead and blue catfish, yellow bass, bowfin, and chain pickerel.

QA/QC: Quality assurance/quality control

Reservoirs

> **Surface/Shallow discharges**: Reservoirs with outfalls that discharge at the surface or within the littoral zone.
>
> **Deep Discharges**: Reservoir with outfalls that discharge outside of the littoral zone.

Rivers: Free-flowing streams with a catchment area ≥ ~150-200 mi^2, presence of low-head dams in the system may occur, most areas are non-wadeable with some areas that may be wadeable and a mean average thalweg depth greater than 1 meter.

Sample Reach: A designated area where sampling may occur. This area is adjusted to account for waterbody type.

Small Streams: Perennial streams that are relatively shallow, narrow and with a catchment area of < 20 mi^2. All of the sample reach is wadeable.

Sub-reach: A section of the sample reach designated for collecting samples.

Wadeable: Stream locations that can easily be traversed on foot and efficient sampling can be performed without the need of a boat.

Personnel Qualifications/Responsibilities

Individuals conducting fish tissue collections shall possess the required state collection permits from the Kentucky Department of Fish and Wildlife Resources. Those persons collecting in waters containing federally threatened or endangered species shall seek guidance from the U.S. Fish and Wildlife Service regarding required federal permits. Field personnel must have an understanding of aquatic organisms and their habitats and be able to properly identify the target species. Field personnel should also be experienced in using electrofishing and other fish sampling equipment, including all safety considerations.

Site Selection

The sampling reach distance is determined for each waterbody type. Sample reaches will be broken into multiple sub-reaches that shall be delineated before sampling begins. Sub-reaches will begin with Sub-reach #1, the reach that is closest to and located downstream of the outfall, and will proceed to the last sub-reach (the reach that is farthest from the outfall). Fish are to be collected from each successive sub-reach until the target fish samples (see target species section) have been collected. Every effort shall be made to obtain target samples from Sub-reach #1. If the target samples cannot be obtained within Sub-reach #1, field personnel shall proceed to Sub-reach #2 and then to the next sub-reach until the target samples are collected. Field personnel shall continue using successive sub-reaches, up to the maximum (defined below), until the target fish samples are obtained. Fish from multiple sub-reaches may be combined to complete the target sample. However, each sub-reach shall be extensively sampled before moving to the next sub-reach, which could require multiple passes through a sub-reach. Before starting a second pass through a sub-reach, sufficient time shall be allowed to let fish settle back into natural habitats and the water to clear.

In the event an effluent-receiving stream length is less than the prescribed sub-reach length, the sub-reach length shall be extended into the subsequent receiving stream downstream of the confluence with the receiving stream. Every effort shall be made to collect fish from the available habitat of the effluent-receiving stream before moving to the downstream receiving stream. However, when the target fish sample is not present in the effluent-receiving stream, the collection effort is extended into the next receiving stream. That collection effort will continue at the effluent-receiving stream's confluence with the receiving stream with sampling conducted in successive downstream sub-reaches. When necessary for composites, any target fish collected in the effluent-receiving stream will be combined with fish from the receiving stream to complete the composite.

NOTE: All stream-size designations are approximate and best professional judgment should be used when determining stream type when a site catchment area is close to two stream types (i.e. small and large streams). The fish community composition should be used to help make this determination.

NOTE: Sample strategies presented are for electrofishing. Sampling with other gear (seines, gill nets, trot lines and/or hook and line) may be necessary. If sampling strategies are altered, sampling closest to and downstream of the outfall is required.

Small Streams

The sample reach will be located immediately below the effluent outfall or drainage ditch in an effluent-receiving stream. Ephemeral streams and effluent drainage ditches are not included in the sample reach. In this context, ephemeral stream refers to those sections of channel immediately downstream of an outfall that only have surface flow during outfall discharge or high runoff conditions after precipitation events. The sample reach will extend downstream for a total length of no more than 400 meters. This 400-meter sample reach shall be broken into four 100-meter sub-reaches, with Sub-Reach #1 being the most upstream sub-reach (i.e. the sub-reach closest to the effluent outfall) and Sub-reach #4 being the most downstream sub-reach (Figure 1). Fish shall be collected from each sub-reach, starting at Sub-reach #1 and progression toward and ending at Sub-reach #4, until the target samples have been collected. Collection shall commence at the downstream end of each Sub-reach and progress in an upstream direction. Samples shall be collected from the entire width of stream.

If at the time of sampling, surface water is not flowing, and there are obvious breaks in stream connectivity, (*i.e.* pool habitat with dry channel above and/or below,) annotations must be made on a data sheet indicating no flow and site photographs must be taken to document current conditions. Fish collection activities will then be canceled, and rescheduled within the prescribed compliance sampling period. If fish tissue cannot be collected or are not available, the 5.0 µg/L water column limit shall apply.

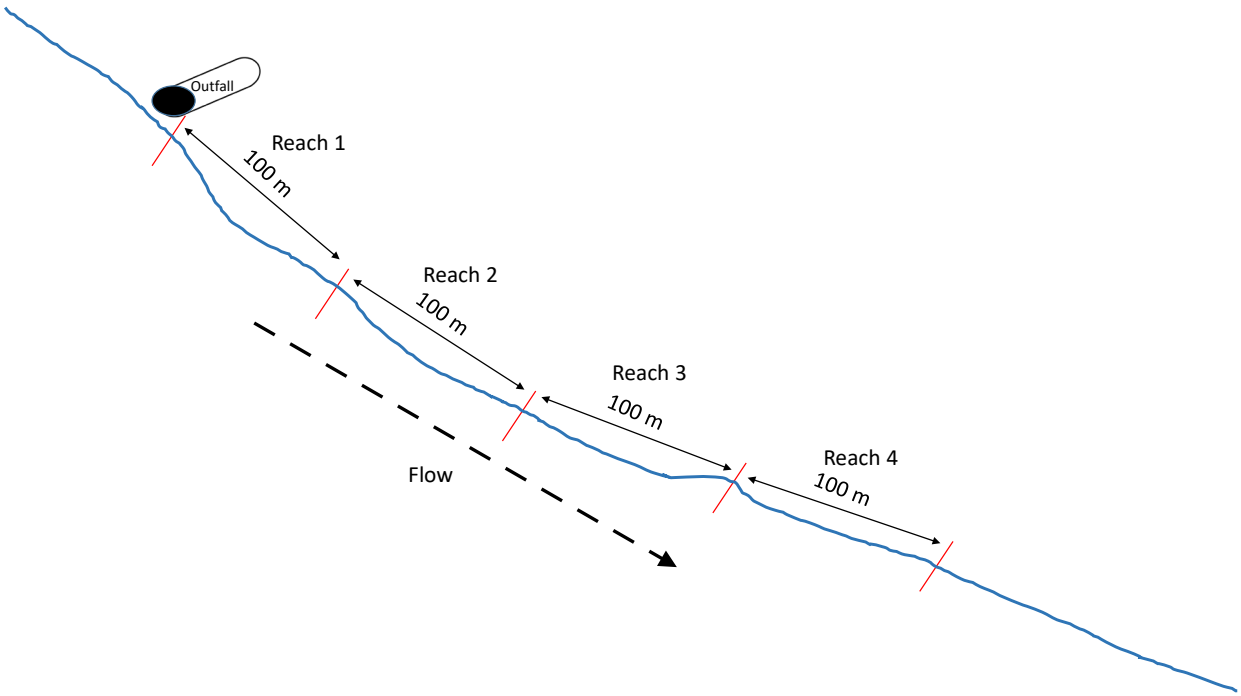

Figure 1. Generalized sub-reaches within a sample reach for small streams.

Large Streams

The sample reach shall be located immediately below the effluent outfall or drainage ditch in the effluent-receiving stream. Ephemeral streams and effluent drainage ditches are not to be included in the sample reach. The sample reach shall extend downstream for a total length of 500 meters. The sample reach for large streams is 100 meters longer than the sample reach for small streams to provide more sampling area to find the appropriate habitats needed to collect the target fish sample. This 500-meter sample reach shall be broken into five 100-meter sub-reaches, with Sub-reach #1 being the most upstream sub-reach (i.e. the sub-reach closest to the effluent outfall) and Sub-reach #5 being the most downstream reach (Figure 2). Fish shall be collected from each sub-reach, commencing at Sub-reach #1 and progressing toward Sub-reach #5, until the target fish samples have been collected. Collection shall commence at the downstream end of each Sub-reach and progress in an upstream direction. Samples shall be collected from the entire width of the stream if the mean width of the stream is <25 meters. In streams with mean widths >25 meters, fish shall be collected in a sampling zone on the bank where the outfall is located. In these circumstances the sampling zone extends from the edge of the water to the center of the stream or to a depth of 1-meter, whichever one occurs first.

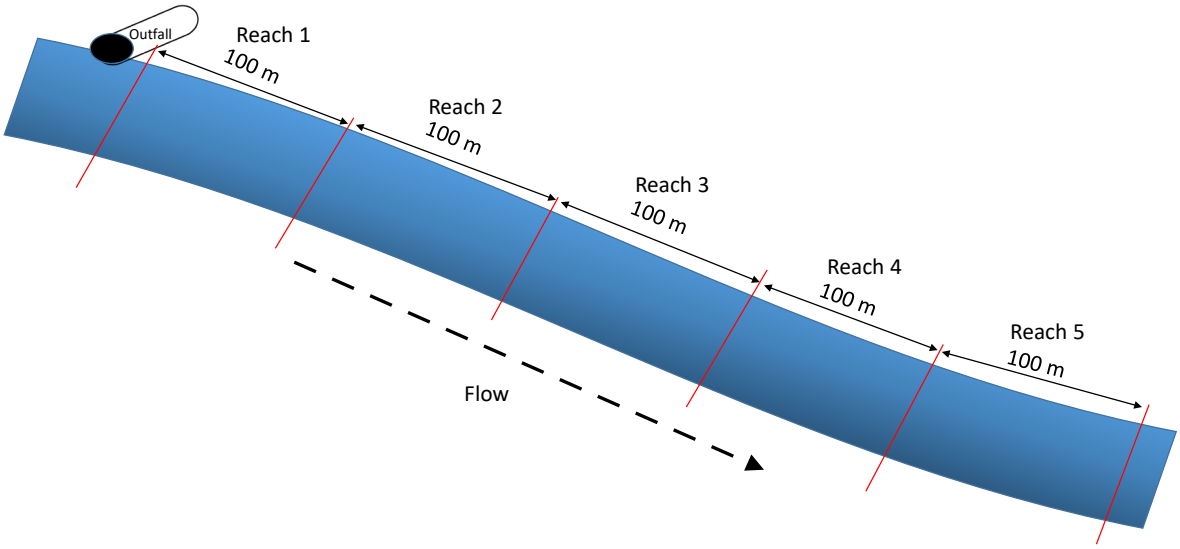

Figure 2. Generalized sub-reaches within a sample reach for large streams.

Rivers

The sample reach shall begin immediately below the effluent outfall or the confluence of the effluent drainage ditch into the effluent-receiving stream. Ephemeral streams and effluent drainage ditches are not to be included in the sample reach. The sample reach shall extend downstream for a total length of 1000 meters. The sample reach for rivers is larger than the sample reach for small and large streams because sampling of these non-wadeable rivers

requires boats where considerably more area might be needed to collect samples. This 1000-meter sample reach shall be divided into two 500-meter sub-reaches, with Sub-reach #1 the most upstream reach and Sub-reach #2 the most downstream reach (Figure 3). Fish shall be collected from each sub-reach, starting at Sub-reach #1 progressing and ending in Sub-reach #2, until the target samples have been collected. Collection shall commence at the upstream end of each sub-reach and progress in a downstream direction. Samples shall be collected in the littoral zone on the bank where the outfall is located.

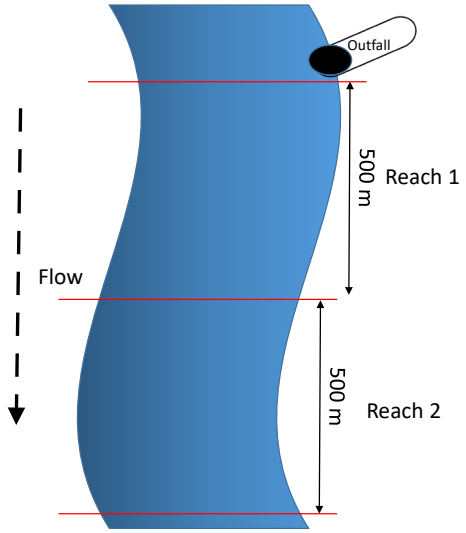

Figure 3. Generalized sub-reaches within a sample reach for rivers.

Reservoirs – Surface/Shallow Discharges

The sample reach for reservoirs shall be 3000 meters. Three sub-reaches will be established: 1) Sub-reach #1 shall be located at the effluent outfall and extend 1000 meters downstream of the effluent outfall; 2) Sub-reach #2 shall be established upstream of the effluent outfall; and 3) Sub-reach #3 will begin immediately downstream of Sub-reach #1 and extend 1000 meters downstream (Figure 4). Ephemeral streams and effluent drainage ditches are not to be included in the sub-reach. Sub-reaches for reservoirs are longer than for streams and rivers because many littoral areas of reservoirs can contain unsuitable habitat for targeted fish species and inaccessible areas (e.g. private boat docks, etc.) so the sub-reaches are larger to ensure adequate sampling area.

The order of sampling shall be Sub-reach #1, Sub-reach #2, and then Sub-reach #3. Sampling shall commence in each reach at the point that is nearest the effluent outfall and progress towards the other end of reach. All sampling shall be conducted in the littoral zone.

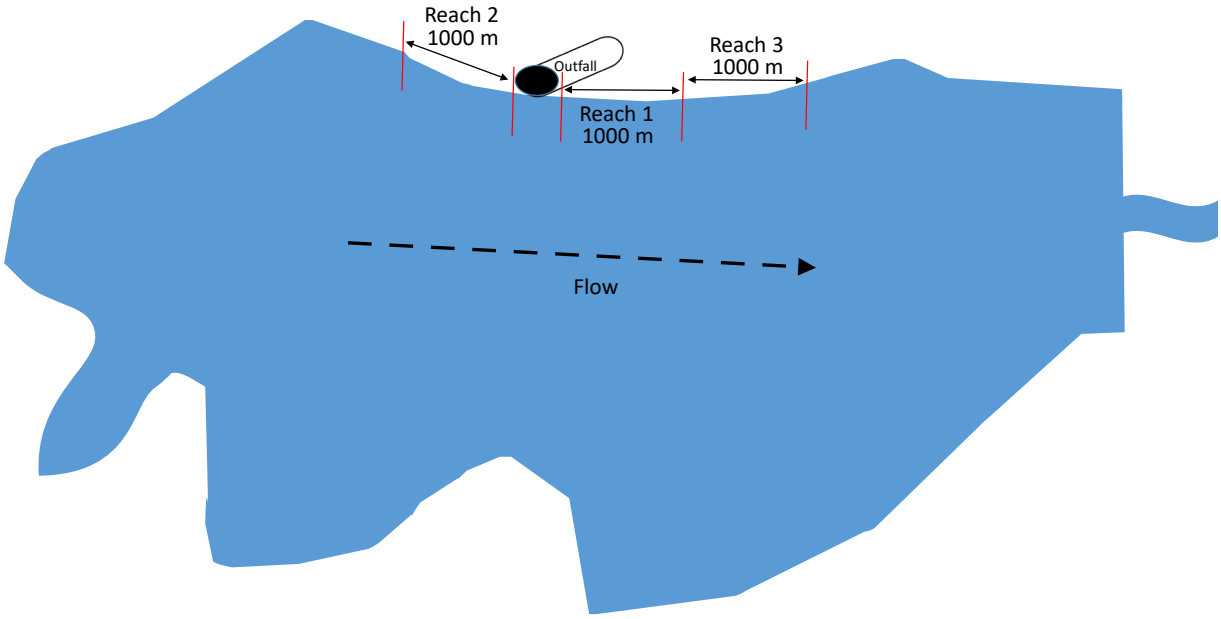

Figure 4. Generalized sub-reaches within a sample reach for reservoirs.

Reservoirs – Deep Discharges

Reservoirs with a deep discharge (a discharge that is not within the littoral zone of the reservoir) are unique situations and require site-specific plans that shall be a requirement of the permit.

Target Species

Small Streams

The following taxa shall be collected: *Semotilus atromaculatus* (creek chubs) (minimum mean composite length of 100 mm). If *Semotilus atromaculatus* are not available, *Lepomis sp*. (Sunfish – minimum 100 mm) or *Campostoma sp*. (Stonerollers – minimum 80 mm), may be used. If *Lepomis sp*. are present, they shall be used in preference to *Campostoma sp*.

Large Streams/Rivers/Reservoirs

The following taxa shall be collected: 1) *Lepomis macrochirus* (Bluegill – minimum mean composite length of 100 mm) and 2) the largest representative predator fish, which would be considered to be the oldest available predator.

If *Lepomis macrochirus* are not available, other *Lepomis* taxa (Sunfish) may be used (minimum mean composite length of 100 mm). Any of the predator taxa listed on page 4 of this document may be used for the predator sample. If a predator fish is not available in these waterbodies, *Ambloplites rupestris* (Rock Bass) (minimum length of 100 mm) may be used.

Qualifying Samples/Target Sample Size

Qualifying Samples

Composite samples shall meet the following requirements:

- All specimens composited shall be of the same species;
- Individuals used in a composite shall be of similar size so that the smallest individual in a composite is no less than 75% (with a target of 90%) of the total length of the largest individual;
- Individuals used in a composite sample shall have been collected during the same sampling event; and
- Provide sufficient tissue for laboratory analysis, including applicable QA/QC requirements.

Individual samples for predators must represent the largest/oldest individual collected.

Small Streams Target Sample Size

- Ten *Semotilus atromaculatus* (or alternatively *Lepomis* or *Campostoma* spp.) shall be collected and composited into two samples (five fish each) from each sample reach. The mean fish length of individuals in each composite shall be ≥100 mm (*Campostoma spp.* ≥80 mm). Furthermore, individuals selected should represent the largest/oldest individuals collected.

Large Streams/Rivers/Reservoirs Target Sample Size

- Five *Lepomis macrochirus* (or other *Lepomis* taxa) shall be collected and composited into a single sample from each sample reach. The mean fish length of individuals in each composite shall be ≥100mm. Furthermore, individuals selected should represent the largest/oldest individuals collected.
- One predator shall be collected from each sample reach (predator sample must represent the largest/oldest individual collected).

Field Collecting Procedures

Fish shall be collected using standard fisheries collection techniques using gear that is appropriate for the targeted species. Electrofishing is the preferred method of sample collection, although other methods (i.e. seines, gill nets, trot lines and/or hook and line) may be necessary.

Collected fish shall be transferred frequently from dip nets to a bucket of water to lessen stress and mortality. In addition, water in buckets or live wells shall be changed periodically to reduce stress and mortality. Aerators can also be employed in buckets and/or live wells to reduce the stress on collected fish.

Sample Field Processing

Once a target sample is collected, the processing procedure can begin. A sterile (i.e. unused) Whirl-pack bag or other plastic zip top bag shall be used to contain each sample.

Field data shall be complete and legible and entered on a chain-of-custody sheet and on the sterile Whirl-pack bag or plastic zip top bag. The following information should be recorded: station #, KPDES permit #, waterbody name, narrative location, upstream/downstream sampling latitude and longitude (resolved to five decimal places), county, date, time, species collected, name(s) of collectors, type of sample (individual/composite) and sample type (field sample). See example in Appendix 1 for a chain of custody.

All fish retained for analysis shall be photographed and lengths of all individuals should be documented. Photographs shall be captured so that each individual fish can be identified from the photograph(s). A ruler should also be included in the photos so the size of each fish is documented. As needed, multiple photographs should be provided to ensure proper documentation of fish species and organism size.

Fish retained for analysis shall be placed in individual sterile Whirl-pack bags or plastic zip top bags. Composite samples may be placed in the same sterile Whirl-pack bag or plastic zip top bag. Fish should be rinsed in native water before placing in Whirl-pack bag or plastic zip top bag if any foreign material (*i.e.* sticks, leaves or algae) is attached to the outside of the fish.

Once all fish samples have been selected and placed in sterile Whirl-pack bags or plastic zip top bags, the samples shall be kept on ice in a cooler until transported to a freezer for long-term storage. The maximum holding time on ice in a cooler is 48 hours.

Field Quality Assurance/Quality Control

A field crew will consist of at least one person who is knowledgeable in the identification and nomenclature of Kentucky fishes. After sampling has been completed, all sampling gear will be thoroughly cleaned to remove all fish so that no fish are carried to the next site. The equipment shall be examined prior to sampling at the next site to ensure that no fish are present. At each site, all collection equipment (*i.e.* nets) that come into contact with fish shall be rinsed in native water.

When delivering a sample to the laboratory for processing, the proper chain-of-custody sheet that corresponds with the sample must be delivered to the laboratory at the same time. Chain of custody records shall be maintained and provided upon request from the Division of Water.

Homogenization

Once fish are collected and delivered to the laboratory, tissue samples shall be prepared by following DOWSOP0300032, *Standard Operating Procedure for Preparation and Homogenization of Fish Tissue Samples*, which is available online at: https://eec.ky.gov/Environmental-Protection/Water/QA/Pages/default.aspx. Tissue sample preparation shall occur in a laboratory environment and not in the field. Predator or bottom feeder fish that exceed twelve (12) inches in length can be processed by utilizing methods for

resection of fish fillets, which will result in a skinless, boneless fillet. Whole body samples shall be processed using all body parts including bones, body liquids, and scales and include all stomach contents.

Selenium Analysis

Analytical test methods and procedures shall be selected from those approved by the U.S. EPA for the detection of total selenium. Approved methods are found in 40 C.F.R. §136.3. Each analytical laboratory must be certified by the Division's Laboratory Certification Section.

Quality Assurance

Below are some recommended quality assurance checks to assist in evaluating the accuracy, precision, and representativeness of the analytical results.

- Additional aliquots of the homogenized fish samples can be analyzed by the laboratory and compared to the original results.
- Photographs of the fish samples may be reviewed to confirm that samples consisted of the correct species with the appropriate lengths.

Results may vary based on a few factors. For example, female fish may have higher concentrations during spawning season. If eggs are observed, annotations should be made on the data sheet. For composite samples, the number of individuals with eggs in the sample should be noted. This information will be helpful in understanding variability between different sampling periods. Other causes of variation in results potentially stem from length/weight/age variation in composite or individual samples.

Records Retention

Field datasheets, copies of field notebooks, voucher photographs, chains of custody, lab reports (including any quality assurance/quality control results), and other related documentation shall be maintained for five years and shall be provided to the Division upon request.

APPENDIX 1. SELENIUM FISH TISSUE CHAIN-OF-CUSTODY

Station #	**Date:**	
Waterbody:	**Start Time:**	**Finish Time:**
Location:	**Upstream**	**Downstream**
County:	**Lat:**	**Lat:**
KPDES Permit#:	**Long:**	**Long:**

Sample Type	County	Sample ID	Sample Description	Species	Length (mm)	Collection Method	Collection Date/Time	LAB Report #
						☒ Composite ☐ Grab		
						☒ Composite ☐ Grab		
						☒ Composite ☐ Grab		
						☒ Composite ☐ Grab		
						☒ Composite ☐ Grab		
						☐ Composite ☒ Grab		

Sample Type: FS=Field Sample, FD= Field Duplicate, LSP=Lab Split

Shipment Temp:

Samples Collected By:

Relinquished by: _____ Date: _____ Received by: _____ Date: _____

Representing: _____ Time: _____ Representing: _____ Time: _____