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Practical Considerations for Sampling

A REAGECON TECHNICAL PAPER

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Abstract

Poor sampling has a profound negative effect on the quality of an analytical result. Incorrect choice of sampling equipment, containers, storage and transport all have a deleterious effect on the sample integrity and thus affect the final result. Sampling errors can be costly because they lead to unnecessary costs in terms of materials, labour and poor decisions made based on the analytical result. This paper outlines practical considerations which should be borne in mind when sampling. These include such aspects as the correct choice of sampling equipment, container types, safety, labelling, transport and storage of samples. It provides a definite set of guidelines on how different sampling errors can be reduced or eliminated thus giving the user greater confidence in the results obtained from their samples.

Keywords: Sample, Sampling, Sampling plan, Equipment, Container, Preservation, Transport

1 Introduction

In analytical practice the importance of sampling cannot be over-emphasised. No matter how carefully analyses are carried out the results will be of limited value unless the sample taken for analysis is collected, preserved, transported and stored in such a way as to ensure its integrity. The use of inappropriate sampling equipment, sample containers, preservation methods and transport/storage conditions can all have an deleterious effect on sample integrity, which will in turn effect the quality of the analytical results.

In recent years sampling has been highlighted and accorded the status it deserves in analytical chemistry. As analytical methodology has improved the use of smaller test portions has become a feature of modern instrumentation. This in turn has made the acquisition of a representative sample more challenging.

The objective in sampling is to produce a sample whose physical and chemical properties are representative of the whole. In addition, it should be as consistent as possible with other samples which would be obtained if the entire sampling protocol could be repeated[(1)]. Sampling errors can be costly because they lead to unnecessary costs in terms of materials, labour and poor decisions made based on the analytical result.

The types of sampling errors which can occur can be classed into 4 main categories:

- Contamination – the addition of extraneous material or molecules to the sample prior to analysis.
- Loss – the reduction in quantity of analyte of interest after sampling.
- Unintentional mistakes – the sample integrity can be compromised by operational or analytical measurement errors.
- Intentional tampering - deliberate interference with the integrity of the sample.

It should be noted though that even when sampling is performed correctly there will still be errors in the final analytical result. The uncertainty of measurement associated with the analytical result is a combination of the uncertainties from the sampling process and from the analysis. As a general rule, uncertainty in analysis constitutes about one third of the total uncertainty incurred by sampling and analysis[(2)]. This means that sampling accounts for two-thirds of the uncertainty associated with any given analytical result. An understanding of the measurement uncertainties which arise from both the analysis and sampling can help to reduce these errors and improve the accuracy of the final analytical result.

2 Sampling plans

To reduce the occurrence of errors, sampling should always be performed in accordance with a well defined sampling plan. There is a wealth of information available on the design of sampling plans[(3-5)]. The sampling plan should be comprehensive and clearly documented to reduce sampling uncertainties to an acceptable level,

bearing in mind the final decision to be made on the results. In short, it should be as inclusive as possible to cover all aspects of sampling, yet be practical enough to adhere to in use. Many approved analytical methods have specified sampling regimes associated with them to ensure uniformity of the techniques used [6-7].

Due to the nature and variety of samples it is impossible to design one plan to suit all needs. For example, liquid samples are taken from a wide variety of environments such as surface water, physiological fluids and industrial waters. In each of these cases the reasons for sampling will be different. An overview of the factors which must be considered when designing a sampling plan are outlined in Figure 1[8].

Once the objectives have been defined the sampling plan is designed to ensure that the information required by the objectives of the measurements are met. A well designed sampling plan should provide answers to the following questions[2]:

- What do we want to know?
- Why do we need this information?
- What happens to the results?
- What actions will follow?

If due attention is paid to these factors it will lead to the design of a good sampling plan which will ensure that the user can have confidence in the final analytical result obtained from the samples.

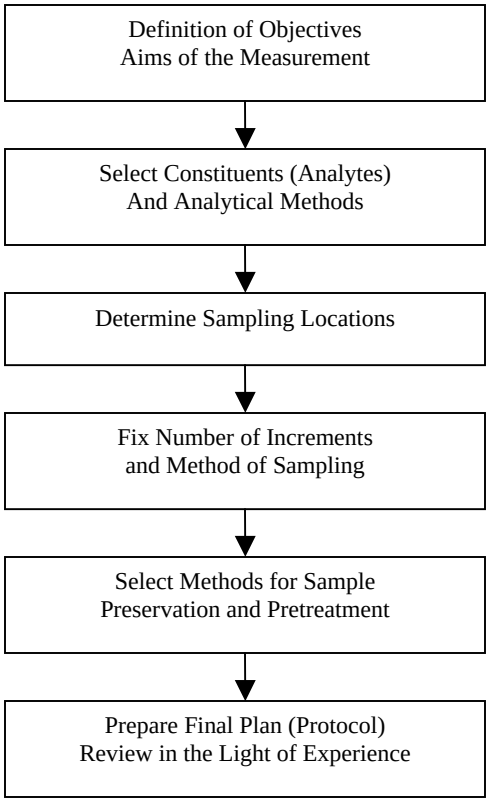

Figure 1. Sequential consideration of factors involved in the design of a Sampling Plan[8]

3 Considerations for sampling

In spite of the amount of information which is available on the design of sampling plans, in many cases it is strictly theoretical and may not be very practical in use[1, 3, 4]. As outlined in Figure 1 there are a variety of points which must be considered when deciding on a sampling strategy:

- The nature of the analyte to be determined
- The analytical method to be used
- The sampling methodology to be used
- Locations for sampling
- The quantity and size of sample required
- The choice of sampling equipment
- Training of sampling personnel
- Choice of sampling containers
- Is some form of sample pretreatment required?
- Are sample preservation techniques required?
- How will the sample be transported or stored prior to testing?
- The sample chain of custody
- Sampling safety

Each of these topics will now be discussed in more detail.

3.1 Nature of analyte

One of the key elements which must be considered when devising a sampling plan is the physical and chemical properties of the analyte of interest. For example, the following properties will all have an influence on the design of the plan:

- Volatility – is the analyte a volatile organic component which could escape into the headspace of a sample container on prolonged storage?
- Light sensitivity – can the sample undergo photo-oxidation of any chemical constituents present, or will increased biological growth lead to a reduction in analyte content?
- Thermal liability – if the analyte is heat sensitive could it be destroyed by raised temperatures due to incorrect transport or storage?
- Chemical reactivity – does the sample contain free chlorine which could react with the analyte?
- Biological degradation – could continued biological growth lead to a reduction in analyte concentration and introduce metabolic waste products?
- Heterogeneity of sampling environment – is the sample source homogeneous or is the analyte concentrated in a particular place (such as a surface layer or sediment)?

3.2 Analytical method

For many analytes there are test methods which are specified by regulatory bodies such as the EPA and ASTM[6, 7]. For sampling which is not covered by a regulatory body it is preferable to use analytical methods which are well established. The analytical method used may require specific sample pretreatment or sample size, thus this will impact on the design of the sampling plan. In all cases any analytical technique used should be validated to determine the accuracy and precision of the method.

3.3 Sampling methodology

For many analytes the sampling methodology chosen will be in accordance with established or specified protocols[6, 7]. For environmental samples the most common sample types taken are as follows[9]:

- Discrete samples – these provide a snapshot image of the prevailing conditions at the time of sampling.
- Simple composite samples – this is where individual volumes are taken at constant time intervals and collected in one container.
- Sequential composite samples – individual samples are taken at a predetermined interval into individual containers.
- Flow proportional sampling – this is collected depending on the water flow during the sampling in such a way that it represents the average conditions of sampling.

- Passive sampling – these are often used in aqueous environments for sampling organic contaminants.

Irrespective of the sampling methodology used, it is important to be aware of the drawbacks associated with each method. As discrete (or grab) samples give a snapshot of the conditions during sampling they may not be truly representative if the sample matrix is heterogeneous. Composite samples may be preferable to discrete samples in the following instances:

- They permit the testing of samples taken from various locations to determine if the analyte of interest is present
- It can reduce the cost of analysing a large number of samples

There are however disadvantages to composite sampling:

- Any possible interactions among analytes or organisms in the sample must be considered.
- When testing multiple analytes the information regarding the analyte relationships in individual samples will be lost.

Despite these disadvantages, if all considerations are evaluated during the planning stage they can be documented and accommodated in the sampling protocol.

3.4 Sampling locations

The selection of the physical locations for sampling will be dependant on the objectives of the sampling plan. In many cases the choice of sampling site is usually selected on the purpose for the sampling process[10]:

- Judgemental sampling - sampling is performed at a specific location (e.g. a pipe outlet in a river) to determine the presence or absence of the analyte.
- Systematic sampling – sample sites are chosen systematically to give a detailed picture of the conditions at the area of interest. Samples are drawn from prescribed points along horizontal and vertical transects.
- Random sampling – sample sites are chosen at random throughout the area of interest.

The locations associated with each type of sampling must be considered in advance of the physical act of sampling to determine what impact it will have on the equipment or procedures used.

It should be noted though that there may be occasions when it may be preferable to perform analysis in-situ using either portable or on-line measuring equipment. Examples of such instances include:

- When the analyte is a physical property of the environment under investigation e.g. flowrate in a stream
- The nature of the analyte is such that it must be tested immediately as it can change if removed from the environment e.g. pH. conductivity
- Continuous monitoring is required for real time process control.
- The cost of analysis of the number of samples required makes it economically sensible to use on-line monitoring equipment.

When using portable or on-line measurement equipment the sampling plan actually becomes the analytical plan. As the analyte is tested in-situ considerations such as sample integrity become issues of data integrity. The choice of location for sensor placement reflects the sampling location, as the sensor must be placed in a location where it won't affect the analyte. An example of commonly used on-line measurement is that of conductivity measurement of purified water, where the removal of a sample from the process will result in a change in conductivity due to the absorption of atmospheric carbon dioxide[11].

3.5 The quantity and size of sample required

The volume and quantity of sample to be collected will be determined by the number of analytes to be tested. In general, the quantity of sample taken should be sufficient to allow for any testing repeats, plus extra which can be stored for future reference (if required). Where several analytes are to be determined it may be necessary to collect the total volume in separate

containers to ensure the adequate stability of the different analytes. In addition, the necessity of minimising contact with the air in the headspace of the container must be weighed up against a possible requirement of having to shake the sample prior to testing. A further point to bear in mind is the potential cost of disposal of excess sample (e.g. toxic heavy metal wastes). In short, the sample size taken should be big enough for the entire testing required, but still practical to take, store and dispose of.

3.6 The choice of sampling equipment

There is a wide variety of sampling equipment available to suit different sampling requirements. When selecting sampling equipment for any particular protocol the following points must be borne in mind:

- The sampling device must be constructed of materials which are compatible with the matrix and target analyte, and also the sample containers.
- Carryover between samples from the sampling device must be prevented.
- The equipment selected should be of suitable strength for its intended purpose.
- The possible adsorption of analyte onto the surfaces of the sampler should be investigated prior to sample collection.
- It is important to be aware of any potential influences the equipment will have on the analyte (e.g. vacuum samplers may produce higher results for BOD, COD and suspended solids than peristaltic pumps)
- Detailed cleaning and storage procedures must be included in the sampling plan to prevent cross-contamination of samples.

Contamination of samples by the sampling equipment is always a problem which increases in significance as the analyte concentration decreases. Table 1 shows the type of potential contaminants which can be introduced into the sample from materials used in sampling devices and well casings[12].

Potential contamination from sampling equipment can be investigated by the use of equipment blanks. These are samples of analyte-free media which are used to rinse the sampling equipment, and then tested for the presence of the analyte. They provide proof of the adequate decontamination of the sampling equipment[13].

3.7 Training of sampling personnel

It is important to note that the greatest factor influencing the collection of a representative sample is the skill of the operator taking it. Even for a technique as relatively simple as grab sampling, the results obtained can vary widely depending on the skill of the sampler. Personnel should be fully trained in the correct use of the equipment to ensure that the sampling is performed correctly. The benefits of having trained personnel is that they are able to recognize altered on-site conditions or equipment malfunctions and react accordingly. The level of training required to perform the specific sampling protocol should be documented in the sampling plan

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Material	**Potential contaminants**
Rigid PVC-threaded joints	Chloroform
Rigid PVC-cemented joints	MEK, toluene, acetone, methylene chloride, benzene, THF, vinyl chloride
Flexible or rigid Teflon tubing	None detectable
Flexible polypropylene tubing	None detectable
Flexible PVC plastics tubing	Phthalate esters & plasticizers
Soldered pipes	Tin & lead
Stainless steel containers	Chromium, iron, nickel, molybdenum
Glass containers	Boron, silicon

Table 1: Potential contaminants from Sampling Devices and Well Casings[12]

3.8 Choice of sampling containers

As with sampling equipment, it is of equal importance that the sample is not contaminated in any way by the sample container during transport or storage. Contamination can occur by several means:

- Leaching of contaminants from the container surfaces
- Leaching of chemicals from the container material (either organic or inorganic)
- Adsorption of trace metals or chemical compounds onto container surfaces
- Changes in equilibrium between analytes present in particulate and solution phases

When selecting the sample container there are several points which must be borne in mind:

- The container chosen should ensure that the sample can be handled safely without causing a chemical or biological hazard.
- It must be properly sealed to isolate the sample from the surrounding environment and also to prevent leakage of the sample.
- Pre-rinsing of the container prior to sampling should be performed (except where analyte adsorption to the walls of the container is suspected).
- Containers where analyte adsorption is suspected should receive a solvent rinse in the laboratory to remove all traces of analyte.
- The airspace at the top of the container should be minimised to prevent loss of any volatile components into the headspace or the dissolution of the headspace gases into the sample.
- If there is a requirement for sample mixing prior to testing then some headspace should be left to facilitate shaking.
- Is it necessary to exclude light from the sample?
- Is the container suitable for the desired storage conditions?
- Does the sample container need to be collected in a sterilized container?
- If the sample is likely to be used for litigatory purposes the container chosen should be fitted with a tamper-proof cap.

Once the appropriate container has been selected it is vital that the sample is correctly labelled to prevent misidentification. The information in the sample label should include:

- A unique identifying number and/or a barcode
- Information about the sample type
- The collector's name
- Date and time of collection
- Place of collection
- Details of any sample preservation techniques used

Indelible ink should be used to record all information. For a well-planned sampling regime it may be possible to use pre-printed labels to which the minimum quantity of information is added at the actual time of sampling. All labels should be affixed to the container prior to sampling. Ideally waterproof labels should be used to prevent potential damage in case of spillage. For the same reason, waterproof sheets should be used for recording sampling information.

3.9 Sample pretreatment

It may be necessary to perform some form of pretreatment of the sample prior to the final analysis, especially for water samples. These pretreatment steps may be carried out either during or directly after the sampling process. Such pretreatment steps may be carried out for the following reasons[14]:

- Removal of any interfering components from the sample (e.g. filtration of suspended solids)
- Transfer of the analyte into a specific matrix for which is required for the analytical method (e.g. solvent extraction)
- Preconcentration of the analyte prior to testing. (e.g. ultrafiltration)
- Preservation of the integrity of the sample

For example, if samples are being taken for metal analysis they should be filtered prior to the addition of acid as preservative. This can be done

by filtering the sample directly into the storage container which contains the acid preservative. This pretreatment is necessary to prevent the dissolution of minerals from any suspended matter which may be present in the unfiltered sample.

3.10 Sample preservation techniques

For some sample types, especially environmental samples, it may not possible to analyse a sample immediately after collection. During transport and storage changes in sample composition can be caused by a variety of chemical or physical mechanisms:

- Oxidation or reduction reactions, e.g. the precipitation of metal oxides and hydroxides due to metal ions reacting with oxygen.
- Depolymerisation, this can occur for condensed inorganic phosphates and polymeric silicic acids.
- Changes in pH due to the absorption of carbon dioxide from the headspace leading to decreasing hardness due to the precipitation of calcium carbonate..
- Volatilization of analyte into the container headspace.
- Adsorption of organic analytes onto plastic container surfaces.
- Diffusion of organic compounds such as phthalate esters or plasticizers from the container.
- Biological degradation of analytes due to continued growth of organisms leading to a potential reduction in analytes and the introduction of metabolic waste products.

It should be noted that preservation techniques only retard biological and chemical changes between the time of sampling and the time of analysis; they do not stop them. In addition, a preservation method chosen for one analyte may interfere with another analyte thus it may be necessary to split samples and use different preservation methods. Table 2 shows the applicability of different preservation methods for aqueous samples[15].

Chemical preservatives should only be used when they do not interfere with the analysis. In addition, they should be added to the sample container prior to sample collection thus allowing all sample portions to be preserved as soon as they are collected.

Irrespective of the preservation technique chosen it is important that they are clearly documented in the sampling plan.

Preservation method	Determined substances/parameters	
Cooling to 2-5°C	Organic, total, Kjeldahl and ammonium nitrogen, free and ionised ammonia, nitrates, nitrites, colour, bromides and bromine compounds, BOD, phosphates, iodides, acidity and alkalinity, sulphates, cationic surfactants, smell	
Addition of chemicals	Sodium thiosulphate	Samples containing free chlorine
	Boric acid	Glucose in urine
	Chloroform	Nitrates, nitrites, suspended matter
	Formaldehyde	Non-ionic surfactants
Acidification to pH < 2	H_2SO_4	Ammonium and Kjeldahl nitrogen, free and ionised ammonia, nitrates, COD, permanganate index, dissolved silicates, silica
	HNO_3	Total hardness, metals
Addition of NaOH to pH > 11	Cyanides, iodides, sulphides	
Other	Phenol index ($CuSO_4$ addition), sulphides (addition of zinc or cadmium acetate)	

Table 2: Applicability of preservation methods for aqueous samples[15]

3.11 Sample transport and storage

Under ideal circumstances the sample should be brought to the laboratory for analysis as soon as it has been taken. As this is generally not the case then the transport and storage of samples becomes of vital importance to ensure that the concentration of analyte does not vary significantly between sampling and analysis. If the sample is not stored or transported correctly it can have a detrimental effect on analytes, which will make any subsequent test results meaningless. In addition, there can be a time lag between receipt of the sample at the laboratory and analysis which will also have an influence on the sample integrity.

The maximum holding time for any sample will vary depending on the analyte, the preservation technique used and also the analytical technique. For many environmental parameters the maximum holding time may be specified in the prescribed method. A well designed sampling plan should take account of both transport and storage considerations (see Table 3 for examples of storage conditions for a variety of environmental analytes). As some samples may require refrigeration once collected, provision must be made for this by the use of ice packs and cool boxes on-site. Finally, to prevent thermal degradation samples should not be transported at temperatures above which they have been collected.

3.12 Sample chain of custody

Besides all the above aspects of sampling, it is equally important to make sure that all stages in the process are clearly documented. The ability to trace the possession and handling of a sample from the time of collection to completion of testing is referred to as the chain of custody. Having a documented chain of custody is useful for routine control of samples, but it is essential when the analytical data is being used for regulatory or litigatory purposes.

The major aspects of the chain of custody include:

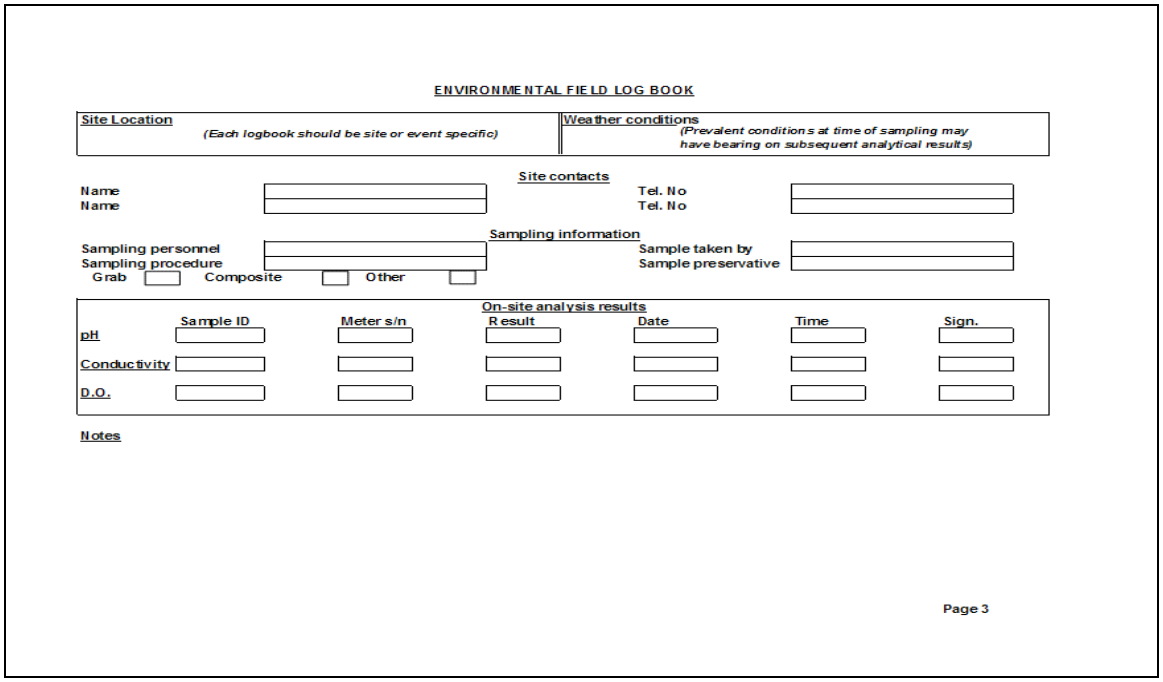
- Sample labels – used to prevent misidentification of samples (see section 3.8 for more details).
- Sample seals – used to detect unauthorized tampering with samples up to the time of analysis. The seal should be attached in such a way that it is necessary to break it to open the sample container, or the sample-shipping container.
- Field Logbook – all relevant information should be recorded in a bound logbook. The information should include sample identification, purpose of sampling, sampling technique, date and time of sampling, preservation type, and time of preservation. All pages should have consecutive numbers to show that nothing has been removed from the log. Any notes made should be objective, factual and free of subjective conclusions (see Figure 2 for an example of an environmental logbook).
- Chain of Custody Record – this should accompany each sample. This record should include the sample number, signature of collector, date, time, and location of sampling, sample type, sample preservation requirements, signatures of persons involved in the chain of possession, and inclusive dates and times of possession (see Figure 3).
- Sample Analysis Request Sheet – this should accompany samples to the laboratory. Upon sampling, the sample collector completes the field portion of the form. The laboratory personnel complete the laboratory portion of the form which includes, name of person receiving the sample, date of receipt, laboratory ID number, and condition of sample (see Figure 4).
- Sample Delivery to Laboratory – the sample should be delivered to the laboratory as soon as possible after sampling.
- Receipt and Logging of Sample – upon receipt the sample condition and seal should be inspected, and the sample logged and stored under the specified conditions until it is assigned to an analyst.
- Assignment of Sample for Analysis – the laboratory manager usually assigns the sample for analysis. The manager or analyst is responsible for the samples' care and custody.

- Disposal – after analysis, and specified retention time, samples are disposed of in a safe manner which should be in accordance with local and national regulations.

Analyte	Container type*	Preservative	Min. vol (ml)	Temp (°C)	Max. holding time
Ammonia	P, BG	H_2SO_4 (pH<2)	200	n/a	48 h
Kjeldahl N	P, G	H_2SO_4 (pH<2)		n/a	48 h
Organic C	G	H_2SO_4 (pH<2)		n/a	24 h
Chlorides	P, G	n/a	100	n/a	15 days
BOD	P, G	n/a	1000	n/a	24 h
COD	P, G	H_2SO_4 (pH<2)	100	n/a	24h
Hardness	P, G	HNO_3 (pH<2)	100	n/a	1 month
Fluorides	P	n/a	300	n/a	7 days
Hydrocarbons	G	CCl_4	300	n/a	7 days
Oils & fats	G	HCl (pH<2)	1000	4	15 days
Mercury	BG	HNO_3 (pH<5)		n/a	1 month
Nitrates	P, G	n/a	100	4	48 h
Nitrites	P, G	n/a	100	4	24 h
Phosphates	P, G	n/a	100	4	48 h
Suspended solids	P, G	HNO_3 (pH<1.5)	1000	4	24 h
Detergents	BG	$CHCl_3$	200	4	24 h
Turbidity	G	n/a	100	4	24 h

Key : P = Polyethylene, G = Glass, BG = Borosilicate glass

Table 3. Examples of different storage conditions for environmental analytes[16]


ENVIRONMENTAL FIELD LOG BOOK

Site Location (Each logbook should be site or event specific)

Weather conditions (Prevalent conditions at time of sampling may have bearing on subsequent analytical results)

Site contacts

Name
Name
Tel. No
Tel. No

Sampling information

Sampling personnel
Sampling procedure
Grab Composite Other
Sample taken by
Sample preservative

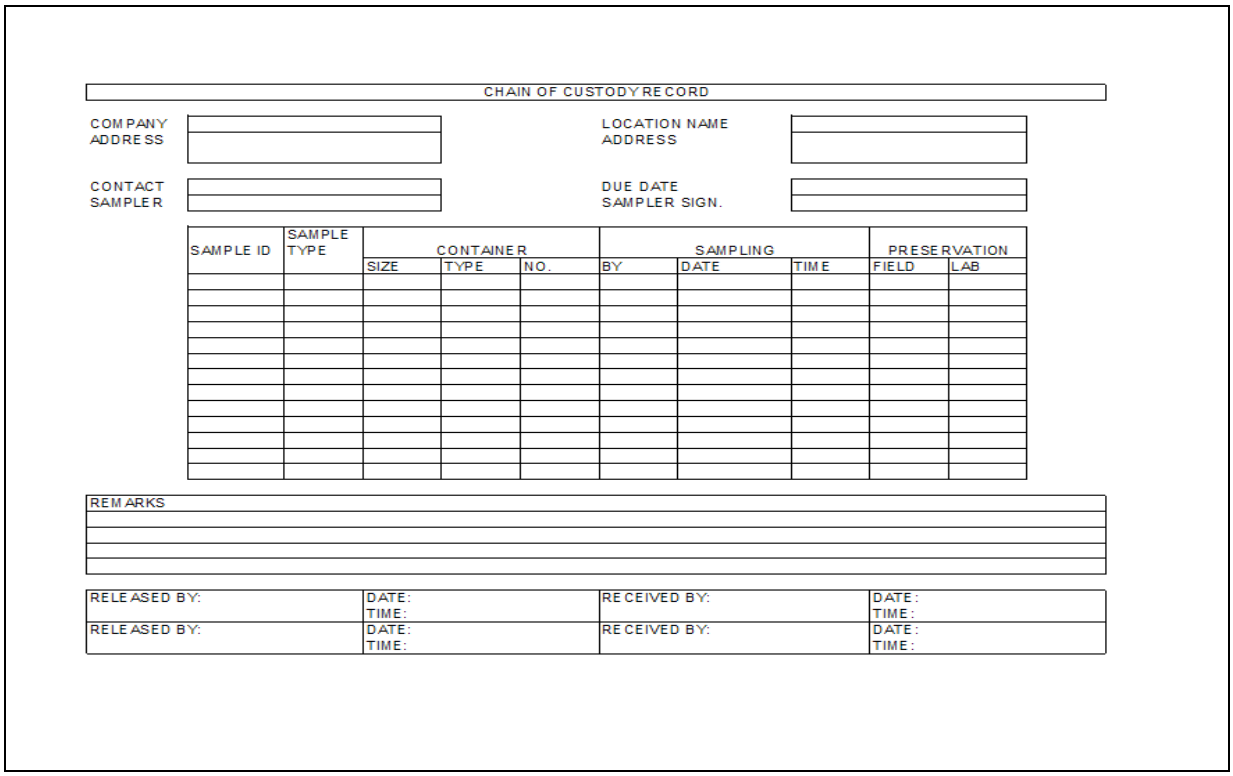
On-site analysis results

	Sample ID	Meter s/n	Result	Date	Time	Sign.
pH						
Conductivity						
D.O.						

Notes

Page 3

Figure 2 Example of environmental sampling field logbook



CHAIN OF CUSTODY RECORD

COMPANY
ADDRESS

LOCATION NAME
ADDRESS

CONTACT
SAMPLER

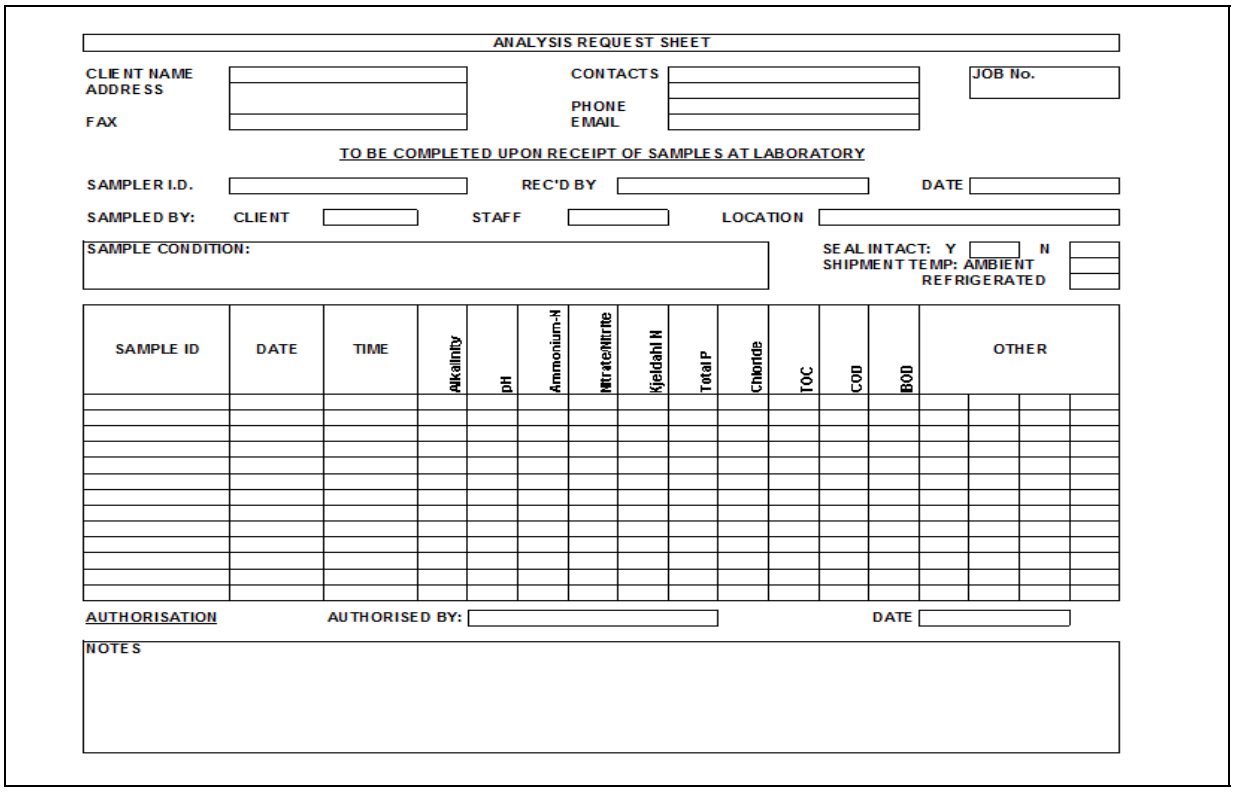
DUE DATE
SAMPLER SIGN.

SAMPLE ID	SAMPLE TYPE	CONTAINER			SAMPLING			PRESERVATION	
		SIZE	TYPE	NO.	BY	DATE	TIME	FIELD	LAB

REMARKS

RELEASED BY:	DATE: TIME:	RECEIVED BY:	DATE: TIME:
RELEASED BY:	DATE: TIME:	RECEIVED BY:	DATE: TIME:

Figure 3 Example of a typical Chain of Custody Record



ANALYSIS REQUEST SHEET

CLIENT NAME
ADDRESS

FAX

CONTACTS

PHONE
EMAIL

JOB No.

TO BE COMPLETED UPON RECEIPT OF SAMPLES AT LABORATORY

SAMPLER I.D.

REC'D BY

DATE

SAMPLED BY: CLIENT

STAFF

LOCATION

SAMPLE CONDITION:

SEAL INTACT: Y N
SHIPMENT TEMP: AMBIENT
REFRIGERATED

SAMPLE ID	DATE	TIME	Alkalinity	pH	Ammonium-N	Nitrate/Nitrite	Kjeldahl N	Total P	Chloride	TOC	COD	BOD	OTHER			

AUTHORISATION AUTHORISED BY: DATE

NOTES

Figure 4. Example of Analysis Request Form

3.13 Sampling safety

The safety of all personnel involved in the sampling process must always be kept in mind when devising any sampling plan. It is important to identify any precautions which should be taken with the analyte in question, and also any hazards associated with the test reagents used. In addition, the physical hazards of the sampling site must be considered. For some protocols, specialist equipment may be required (such as respirators, hard hats, safety shoes, etc[17]). The sampling plan should detail all these requirements, plus any special considerations such as a requirement for two persons for sampling from a boat. It should also outline procedures for the safe handling of sampling equipment and containers, and for the safe transport and storage of samples.

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4 Conclusion

The importance of correct sampling cannot be overstated. The challenge in sampling is to collect, preserve, transport and store a sample without interfering with the integrity of the analytes of interest. If these steps are not performed correctly the resulting errors can be costly because they lead to unnecessary costs in terms of materials, labour and poor decisions made based on the analytical result.

To reduce the occurrence of errors sampling should always be performed in accordance with a well defined sampling plan. Besides detailing the how and where of sampling, the sample plan should also address the choice of sampling container, methods of sample preservation and transport and sample safety. Sampling which is carried out in accordance with such a sampling plan it will lead to improved accuracy in the final analytical result.

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Leo Geary has worked for Reagecon Diagnostics Ltd. since 1998 and is currently the Senior Chemist in the Technical Services Department. In this role, he is involved in the provision of technical support for the complete Reagecon product range to customers to enable them to achieve high quality analytical results. This includes the provision of a traceable calibration and requalification service for all electrochemistry instruments.