

Herring Gull (*Larus argentatus*)

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Contents

1	German Environmental Specimen Bank.....	1
2	Objective of this Guideline	2
3	Function of the Specimen Type	2
4	Target Compartments	2
5	Predefinitions for the Sampling	3
5.1	Species Determination	3
5.2	Selection and Definition of Sampling Sites.....	3
5.3	Selection of Individuals and Sample Size	3
5.4	Sampling Period and Frequency	4
5.5	Area-Related Sampling Scheme	4
6	Sampling Procedure	4
6.1	Required Equipment and Cleaning Procedures.....	4
6.2	Sampling Technique.....	5
7	Biometric Sample Characterization	5
8	References.....	6

Appendices: Checklist to Prepare and Conduct the Sampling Specimen Data Sheets

Guidelines for Sampling, Transport, Storage and Chemical Characterization of Environmental and Human Samples

Status: March 2018, V 2.0.5

1 German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an instrument for environmental monitoring of the

Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) subject to specialist and administrative coordination by the Federal Environment Agency (UBA). The ESB collects

ecologically representative environmental and human samples and stores and investigates them for environmentally relevant substances.

Specific operating procedures as well as the conception of the ESB are the basis of the program. (Umweltbundesamt 2008, 2014)

The long-term storage is carried out under conditions which, as much as possible, exclude a change in state or a loss of chemical characteristics over a period of several decades. The archive therefore provides samples for retrospective investigations of substances for which the potential risk for the environment or human health is not yet known.

Comprehensive information on the ESB is available at www.umweltprobenbank.de.

2 Objective of this Guideline

Sampling is the first and most important step to safeguard the quality of samples and data. It is the result of science-based, standardized methods to avoid contamination and inhibit loss of chemical information. The need for an exceptionally high level of quality assurance results from the extraordinary value of the samples as archive material. Representativeness and reproducibility of the samples are the basis for spatial and temporal comparison.

The current guideline is an update of the Paulus *et al.* (2010) version.

Transport, further sample treatment and storage as well as chemical analysis have to be carried out according to the current guidelines of the ESB.

3 Function of the Specimen Type

The herring gull (*Larus argentatus*) proved to be a good accumulation indicator for marine habitats, representative of the omnivorous trophic level (e.g. Becker 1989, Becker *et al.* 1989, 1991, Elliot *et al.* 1989, Burger and Gochfeld 1995, Kahle and Becker 2000, Weseloh *et al.* 2002, 2006, Gauthier *et al.* 2008, 2009, Helgason *et al.* 2008, Rüdél *et al.* 2010, 2011, Carlsson *et al.* 2011, Nordén *et al.* 2013, Blukacz-Richards *et al.* 2017). Primarily the pollutant content of the eggs is analyzed, which

mirrors the environmental burden situation of the area to be assessed.

The following criteria underline the appropriateness of the use of the herring gull as an indicator organism (q.v. Glutz v. Boltzheim and Bauer 1982, Dervedde 1993, Garthe *et al.* 1999, Kubetzki and Garthe 2003, Olsen and Larsson 2004):

- It is widely spread.
- As a sedentary bird / short distance migrant, it is relatively resident.
- It is continuously available in large numbers. Variations within the population are few, thus monitoring continuity is guaranteed.
- The feeding behavior of the herring gull has been thoroughly investigated. In marine habitats it feeds primarily on fish, crustaceans and mussels.
- The eggs' pollutant content reveals sufficient spatial relation.
- The sampling is relatively easy to perform. The breeding colonies normally have high population densities, so the eggs can be collected in large numbers within a short time.
- There are no general conservation regulations that would constrain the use of this species's eggs for scientific research.
- The species is easy to identify.

4 Target Compartments

Comprehensive studies have revealed that especially liver, kidney, plumage and egg samples are suitable as accumulation indicators. The use of eggs has the advantage that through the determination of biometric characteristics and derived indices (e.g. Ratcliffe's Index) useful information on the effects of chemical substances can also be ascertained. Thus, they can be utilized both as an accumulation and effect indicator. The egg contents serve as an ESB sample for substance investigations.

The following criteria underline the use of eggs as a target compartment when birds are to be used in monitoring studies:

- Eggs have a sufficient biomass.
- Date and location of the egg sample can be precisely defined.

- Eggs mirror the contamination of the hatching females.
- The animals need not be killed.
- The time spent on collection is minimal compared to catch campaigns.
- The eggs are easy to handle during sampling and the sample preparation.
- The shell is excellent protection and inhibits contamination of the sample (egg contents).
- According to the current level of knowledge, the chemical composition of eggs is more constant than of the viscera.
- Eggs constitute an important pathway for the excretion of lipophilic persistent pollutants and some heavy metals.
- In specific stages of development their reaction to toxic chemicals is very sensitive.

When evaluating the analysis data, attention must be drawn to the fact that the ovary builds a sort of barrier to many heavy metals. This barrier inhibits higher concentrations of e.g. lead and cadmium in the eggs.

5 Predefinitions for the Sampling

5.1 Species Determination

Adult herring gulls are relatively easy to identify by their characteristic features (Glutz v. Boltzheim und Bauer 1982, Grant 1986, Olsen and Larsson 2004). The unambiguous identification of their eggs is much more difficult. They are easily mistaken for the eggs of other gull species due to their broad color variability. Hence, a reliable species identification is often only guaranteed in combination with the nesting birds.

The base shell color of the fusiform, circa 70 x 49 mm sized egg of the herring gull is usually light olive green, green or auburn, but can vary from whitish blue to deep rubiginous (Fig. 1). Most of the often black, dark brown or olive drab spots or dots are developed. An irregular scribble is unusual. Moreover, dense markings and scanty mottling occur. Eggs without markings are uncommon (Harrison 1975).



Fig. 1: Color varieties of herring gull eggs (Optimedia 1998)

5.2 Selection and Definition of Sampling Sites

The selection of sampling sites is primarily determined through the occurrence of breeding colonies in the sampling areas. Since the sampling sites must be representative for the marine ecosystem, the vicinity of local emissions sources must be avoided.

The breeding colony should be large enough that an adequate statistical fixed number of eggs can be removed without endangering the population through the sampling.

5.3 Selection of Individuals and Sample Size

Usually, the herring gull clutch consists of two to three eggs. Since fresh eggs should be sampled from each clutch, only the second egg is gathered. This sampling approach also allows for the classification of the date of lay.

For the description of one sampling site, a random sample number of at least 25 eggs should be reached. With this random sample number the biometric as well as the analytical variability of the egg samples is sufficiently taken into account. 25 eggs with an average egg content of 70 g multiply to a total sample quantity of at least 1,700 g of egg contents. With this minimum number, the ESB required quantity of 1,100 g of egg contents is reached. 35 eggs should be collected per sampling site in order to reject incubated or damaged eggs.

5.4 Sampling Period and Frequency

The sampling of herring gull eggs is carried out during the main nesting period (April/March). The removal of the eggs is restricted to a limited time span of 3-5 days.

5.5 Area-Related Sampling Scheme

Based on the sampling guidelines, specific definitions for the individual sampling areas and sites must be made and documented in an area-related sampling scheme. These include, but are not limited to:

- location and demarcation of the sampling sites,
- required sample size (depending on the weight of the eggs in the specific breeding colonies),
- appropriate authorities,
- supporting ornithological groups.

Here it is important to consider how to ensure a long-term sampling continuity. If changes are made, the document must be updated.

6 Sampling Procedure

All data collected during sampling and biometric sample characterization must be documented in the corresponding specimen data sheets (see appendix). In addition, a protocol must be prepared for each sampling with the following information:

- persons that participated in the sampling,
- chronological sequence of the sampling,
- the underlying version of the sampling guideline and the area-related sampling scheme for the current sampling as well as,
- deviations from the sampling guideline and the area-related sampling scheme.

6.1 Required Equipment and Cleaning Procedures

Field Work:

- dowels to mark the clutches,

- pencil (soft) for numbering the eggs (no felt pen because of possible contamination with its contents),
- egg carton for keeping the eggs safe during sampling and the interim storage,
- cooling device ($5 \pm 2^\circ\text{C}$) for interim egg storage and transportation,
- specimen data sheets for documentation of sampling data.

Laboratory:

- cooling device ($5 \pm 2^\circ\text{C}$) for the storage of the eggs until further processing,
- glass beaker with deionized water for the determination of the incubation stage,
- disposable gloves,
- tissues to clean the eggshells,
- clean bench with particle and activated carbon filtration,
- stainless steel containers (5.5 l) with lids and fasteners,
- stainless steel scalpel to open the eggs,
- stainless steel sieve,
- Petri dishes to dry the eggshells,
- identity cards to label the Petri dishes,
- scales (reading 0.1 g) to determine the weight of the fresh egg,
- precision scales (reading 0.001 g) to determine the weight of the dry shell,
- caliper (reading 0.1 mm) to determine the size and diameter of the egg,
- micrometer caliper (reading 0.001 mm) to measure the thickness of the eggshell,
- cooling device for the storage of the samples in the gas phase above liquid nitrogen (LIN),
- liquid nitrogen,
- specimen data sheets.

For the packing and immediate deep-freezing of the eggs' contents, the stainless steel containers are inserted directly during the sample preparation in the gas phase above liquid nitrogen.

Sample containers and all equipment are cleaned in a laboratory washer using a chlorine-free powerful washing agent in the first step. After cold and hot ($90 - 95^\circ\text{C}$) rinsing, neutralization using 30% phosphorus acid in warm water is performed, followed by hot and cold rinsing with deionized water. After this procedure, the containers are dried in a cabinet dryer at 130°C ($\pm 10^\circ$) for a minimum of an

hour (sterilization). The containers remain in the closed cabinet dryer while they are left to cool. Sterilization is not applied to synthetic materials.

6.2 Sampling Technique

During the first inspection of the breeding colony sufficient clutches with one egg are marked by pegging a dowel. At the same time eggs in the clutches are also marked, to distinguish them from the subsequently laid second egg. During a second inspection, two to three days later, the second egg in the marked clutches is removed. The eggs are numbered in the sequence of their removal using a soft lead pencil and are laid in egg cartons to prevent breakage.

Immediately after the removal, the eggs are temporarily stored in a cooling device (refrigerator) at $5 \pm 2^\circ\text{C}$. An interim storage until further sample processing should not exceed 2 weeks. To prevent the shells from cracking, eggs should not be frozen.

Sample preparation and biometric sample description are carried out in the laboratory. First, the incubation stage is determined using Hays und LeCroy's (1971) method of putting the eggs into a glass beaker filled with deionized water. This guarantees that only fresh eggs are used. Only those eggs which comply with the situation a) to d) (Fig. 2) are considered. After immersion, the eggs are cleaned and dried using tissues to remove dirt particles and water. For biometric sample description, 25 eggs, from which egg content is used as sample, are measured to determine length, diameter, and fresh weight prior to the removal of contents from the calcium shell.

The separation of the calcium shell and the egg content is carried out in clean air conditions. The shell is partly cut open above and below its equator. Then the content of the egg is emptied slowly (approx. five seconds) into a stainless steel sieve. Due to the immediate shock-freezing process, the nitrogen prevents the egg contents from adhering to the container walls. After visual survey of the sample quality (egg yolk without noticeable solid body structures!) the egg contents are gradually transferred to the stainless steel collecting containers also filled with liquid nitrogen.

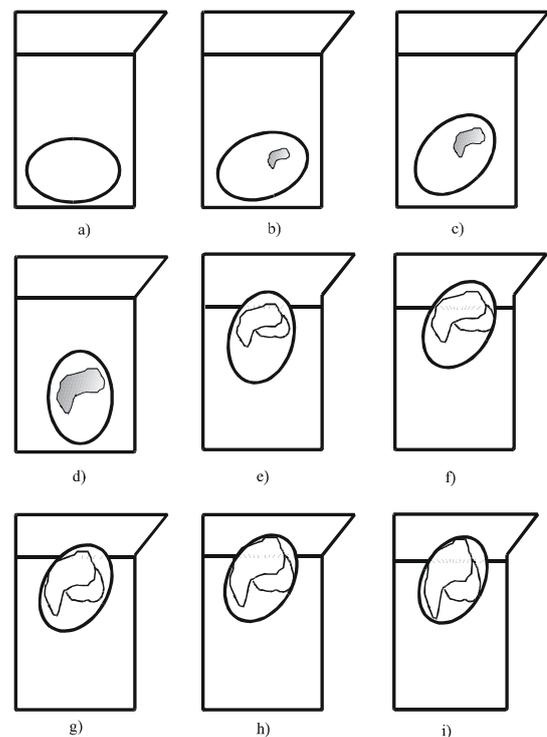


Fig. 2: Incubation stages of birds' eggs (Hays und LeCroy 1971)

The individual fast-freezing of egg content in the stainless steel sieve, prior to the transfer to the sample container provides the advantage – apart from the opportunity for visual survey – of preventing the individual egg content from combining and freezing together, which considerably simplifies the subsequent homogenization. The required amount of nitrogen needed for the eggs depends on the sample quantity. After all the eggs have been transferred to the stainless steel container, the liquid nitrogen must be removed.

After sample preparation the shells are washed again to eliminate residual egg content remaining on the insides of the shells. Then the eggshells are transferred individually to clearly labeled Petri dishes for drying at room temperature. After drying for at least 7 days, the thickness and the weight of the dry shell are determined.

7 Biometric Sample Characterization

For each sampling site a detailed biometric characterization is carried out using the first 25 eggs. The following parameters are ascertained:

- length of the egg (reading 0.1 mm),
- diameter (reading 0.1 mm),
- fresh weight of the egg (reading 0.1 g),
- eggshell dry weight (reading 0.001 g),
- eggshell thickness (reading 0.001 mm).

The determination of the lengths, diameters and fresh weights takes place preceding the separation of egg contents and calcium shell.

After weighing the shells dried in the Petri dishes (eggshell dry weight), the eggshells thickness is determined using a micrometer indicator caliper as follows: Four fragments (both of the pole caps and two parts from the equatorial region) are separated from the eggshell (shell with undamaged membrane). On each of these four fragments five point measurements are carried out. Of the five point measurements an average thickness of the eggshell is derived for the pointed pole and the edgeless pole, from the ten measurements (equatorial region 1 and 2) the thickness of the equatorial region is defined. The average thickness of the entire eggshell is calculated, based on the 20 measurements per egg.

At the edgeless pole, the egg membrane can detach from the eggshell. In this case, the egg membrane must be measured separately at three different points. The average of the three values is added to the measured thickness.

In addition, the Ratcliffe Index has been proven to indicate effect on bird eggs. The index (Ratcliffe 1967, 1970) is calculated as follows:

$$R = \frac{\text{Eggshell Weight [mg DW]}}{\text{Length [mm]} \times \text{Width [mm]}}$$

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Checklist to Prepare and Conduct the Sampling

Specimen Type	Herring gull (<i>Larus argentatus</i>)
Target Compartment	egg content
Individual Specimens	undamaged eggs, laid as second egg (incubation stage a – d according to Hays and LeCroy 1971)
Random Sample Number	at least 25 eggs per sampling site
Sample Quantity for the ESB	1,100 g (35 eggs)
Sampling Period	main incubation period (April/May)
Sampling Frequency	1 sampling per annum
Required Equipment for Field Work	<ul style="list-style-type: none"> • dowels as markers for the clutches • pencil (soft) to number the eggs • egg carton for the safe storage of the eggs • specimen data sheets for documentation of the sampling data
Required Equipment for Laboratory Work	<ul style="list-style-type: none"> • beaker with deionized water for the determination of the incubation stage • disposable gloves • tissues to clean the eggshells • clean bench with particle and activated carbon filtration • stainless steel containers (5.5 l) with lids and fasteners • liquid nitrogen • stainless steel scalpel to open the eggs • stainless steel sieve • Petri dishes with identity cards to dry the eggshells • scales (reading 0.1 g) to determine the weight of the fresh egg • precision scales (reading 0.001 g) to determine the weight of the dry shell • caliper to determine the size of the egg • micrometer caliper to measure the thickness of the eggshell • specimen data sheets
Sample Packing	<ul style="list-style-type: none"> • egg cartons for the eggs, stainless steel containers (5.5 l) for the egg contents
Transport and Interim Storage	<ul style="list-style-type: none"> • cooling device ($5 \pm 2^{\circ}\text{C}$) for the eggs, cooling device for the storage of the egg contents in the gas phase above liquid nitrogen (LIN)
Biometric Sample Characterization	<ul style="list-style-type: none"> • length of the egg (reading 0.1 mm) • diameter of the egg (reading 0.1 mm) • fresh weight of the egg (reading 0.1 g) • eggshell dry weight (reading 0.001 g) • eggshell thickness (reading 0.001 mm) • Ratcliffe's Index

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 1: Sampling Location

Herring gull (*Larus argentatus*)

Identification:

____ / X / ____ / ____ / ____ / ____

_____	Specimen Type
_____	Specimen Condition
_____	Collection Date (MM/YY)
_____	Sampling Area (SA)
_____	Sampling Region (SR)
_____	Sampling Site (SS)
_____	Additional information

Sampling Site (plaintext) _____

Sampling Point (number) _____

Sampling Point (plaintext) _____

Sampling Leader _____

Remarks _____

Notes _____

GERMAN ENVIRONMENTAL SPECIMEN BANK
Specimen Data Sheet 2: Sampling Dates and Storage
Herring gull (*Larus argentatus*)

Identification: ___ ___ / X / ___ ___ / ___ ___ / ___

Sampling Point: ___ ___

Food surplus at the clutch:

Nest material:

Remarks:

Sampling Dates:	1	2	3	4	5	6
Date of the sampling [dd.mm]						
Date of the sample preparation in the laboratory [dd.mm]						
Duration of the interim storage [dd]						
Number of eggs stored						

Storage

Number of Stainless Steel Containers	Weight Empty [g]	Weight Filled [g]	Weighted Sample [g]	Remarks
_____	_____	_____	_____	
_____	_____	_____	_____	
_____	_____	_____	_____	
_____	_____	_____	_____	

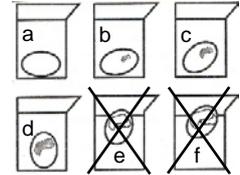
Remarks: _____

GERMAN ENVIRONMENTAL SPECIMEN BANK

**Specimens Data Sheet 3.1: Biometric Parameters – 25 herring gull eggs
Herring gull (*Larus argentatus*)**

Identification:

_____ / X / _____ / _____ / _____



Sampling Point: _____

No.	Date [dd.mm]	Incubation stage a, b, c, d	Length of the egg -- , _ mm	Diameter of the egg -- , _ mm	Fresh weight of the egg -- , _ g	Dry weight of the shell -- , -- g	Thickness of the shell -- -- μm
01							
02							
03							
04							
05							
06							
07							
08							
09							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25							

No. (from to), date, signature of the reviser:

No. (from to), date, signature of the reviser:

Specimen Data Sheet 3.2.1: Biometric Parameters – 25 herring gull eggs

Identification: _____ / X / _____ / _____ / _____ / _____ Sampling Point: _____

Egg no.: 01		X (20) =	
edgeless pole ____ [μm]	pointed pole ____ [μm]	equator 1 ____ [μm]	equator 2 ____ [μm]
1			
2			
3			
4			
5			

Egg no.: 02		X (20) =	
edgeless pole ____ [μm]	pointed pole ____ [μm]	equator 1 ____ [μm]	equator 2 ____ [μm]
1			
2			
3			
4			
5			

Egg no.: 03		X (20) =	
edgeless pole ____ [μm]	pointed pole ____ [μm]	equator 1 ____ [μm]	equator 2 ____ [μm]
1			
2			
3			
4			
5			

Egg no.: 04		X (20) =	
edgeless pole ____ [μm]	pointed pole ____ [μm]	equator 1 ____ [μm]	equator 2 ____ [μm]
1			
2			
3			
4			
5			

Egg no.: 05		X (20) =	
edgeless pole ____ [μm]	pointed pole ____ [μm]	equator 1 ____ [μm]	equator 2 ____ [μm]
1			
2			
3			
4			
5			

Egg no.: 06		X (20) =	
edgeless pole ____ [μm]	pointed pole ____ [μm]	equator 1 ____ [μm]	equator 2 ____ [μm]
1			
2			
3			
4			
5			

Egg no.: 07		X (20) =	
edgeless pole ____ [μm]	pointed pole ____ [μm]	equator 1 ____ [μm]	equator 2 ____ [μm]
1			
2			
3			
4			
5			

Egg no.: 08		X (20) =	
edgeless pole ____ [μm]	pointed pole ____ [μm]	equator 1 ____ [μm]	equator 2 ____ [μm]
1			
2			
3			
4			
5			

Egg no.: 09		X (20) =	
edgeless pole ____ [μm]	pointed pole ____ [μm]	equator 1 ____ [μm]	equator 2 ____ [μm]
1			
2			
3			
4			
5			

Egg no.: 10		X (20) =	
edgeless pole ____ [μm]	pointed pole ____ [μm]	equator 1 ____ [μm]	equator 2 ____ [μm]
1			
2			
3			
4			
5			

Specimen Data Sheet 3.2.2: Biometric Parameters – 25 herring gull eggs

Identification: _____ / X / _____ / _____ / _____ **Sampling Point:** _____

Egg no.: 11		X (20) =		
	edgeless pole ---- [μm]	pointed pole ---- [μm]	equator 1 ---- [μm]	equator 2 ---- [μm]
1				
2				
3				
4				
5				

Egg no.: 12		X (20) =		
	edgeless pole ---- [μm]	pointed pole ---- [μm]	equator 1 ---- [μm]	equator 2 ---- [μm]
1				
2				
3				
4				
5				

Egg no.: 13		X (20) =		
	edgeless pole ---- [μm]	pointed pole ---- [μm]	equator 1 ---- [μm]	equator 2 ---- [μm]
1				
2				
3				
4				
5				

Egg no.: 14		X (20) =		
	edgeless pole ---- [μm]	pointed pole ---- [μm]	equator 1 ---- [μm]	equator 2 ---- [μm]
1				
2				
3				
4				
5				

Egg no.: 15		X (20) =		
	edgeless pole ---- [μm]	pointed pole ---- [μm]	equator 1 ---- [μm]	equator 2 ---- [μm]
1				
2				
3				
4				
5				

Egg no.: 16		X (20) =		
	edgeless pole ---- [μm]	pointed pole ---- [μm]	equator 1 ---- [μm]	equator 2 ---- [μm]
1				
2				
3				
4				
5				

Egg no.: 17		X (20) =		
	edgeless pole ---- [μm]	pointed pole ---- [μm]	equator 1 ---- [μm]	equator 2 ---- [μm]
1				
2				
3				
4				
5				

Egg no.: 18		X (20) =		
	edgeless pole ---- [μm]	pointed pole ---- [μm]	equator 1 ---- [μm]	equator 2 ---- [μm]
1				
2				
3				
4				
5				

Egg no.: 19		X (20) =		
	edgeless pole ---- [μm]	pointed pole ---- [μm]	equator 1 ---- [μm]	equator 2 ---- [μm]
1				
2				
3				
4				
5				

Egg no.: 20		X (20) =		
	edgeless pole ---- [μm]	pointed pole ---- [μm]	equator 1 ---- [μm]	equator 2 ---- [μm]
1				
2				
3				
4				
5				

Specimen Data Sheet 3.2.3: Biometric Parameters – 25 herring gull eggs

Identification: _____ / X / _____ / _____ / _____ **Sampling Point:** _____

Egg no.: 21		X (20) =		
	edgeless pole ---- [µm]	pointed pole ---- [µm]	equator 1 ---- [µm]	equator 2 ---- [µm]
1				
2				
3				
4				
5				

Egg no.: 22		X (20) =		
	edgeless pole ---- [µm]	pointed pole ---- [µm]	equator 1 ---- [µm]	equator 2 ---- [µm]
1				
2				
3				
4				
5				

Egg no.: 23		X (20) =		
	edgeless pole ---- [µm]	pointed pole ---- [µm]	equator 1 ---- [µm]	equator 2 ---- [µm]
1				
2				
3				
4				
5				

Egg no.: 24		X (20) =		
	edgeless pole ---- [µm]	pointed pole ---- [µm]	equator 1 ---- [µm]	equator 2 ---- [µm]
1				
2				
3				
4				
5				

Egg no.: 25		X (20) =		
	edgeless pole ---- [µm]	pointed pole ---- [µm]	equator 1 ---- [µm]	equator 2 ---- [µm]
1				
2				
3				
4				
5				

No. (from to), date, signature of the reviser:

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