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Baseline Standards for Fluid Collections

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Pfc 2018 – Preservation of natural history wet collections
MNHN Paris, 5th - 7th December, 2018

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Baseline Standards for Fluid Collections I

Based on expertise gathered during the

Expert Workshop on Benchmark Standards for the Preservation of Wet Collections

funded by Cloth Makers Foundation (UK) & organised by Chris Collins (NHM, London)

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held at NHM London, 16th - 17th October, 2012

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national museum wales

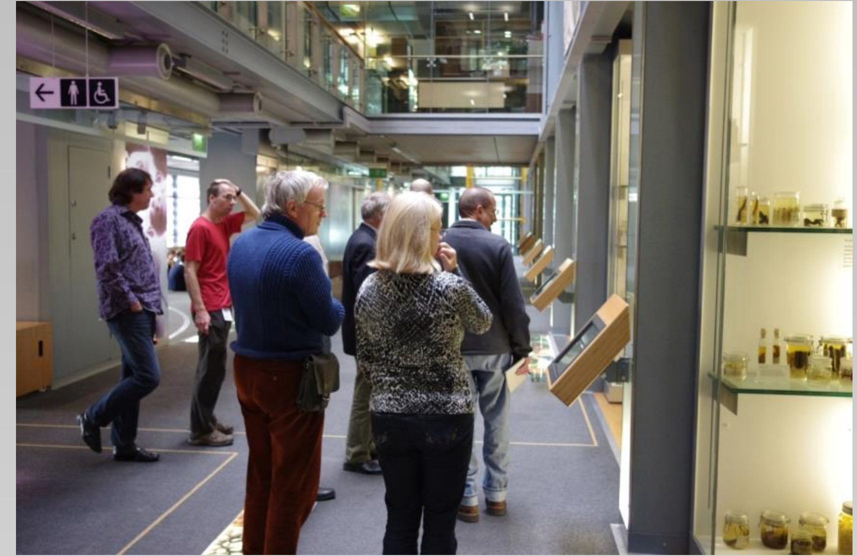
staatliche naturwissenschaftliche sammlungen bayerns

Baseline Standards for Fluid collections – concept and development

- October 2012—Initial meeting at the Natural History Museum (London) with funding from the Cloth Makers Foundation (UK)
- June 2014—Fluid Workshop, SPNHC meeting (Cardiff)
  http://conservation.myspecies.info/node/33
- November 2014—Basic Collections Techniques, Museum für Naturkunde (Berlin)
- March 2016—working meeting, Natural History Museum (London)
- June 2016—Fluid Workshop, SPNHC meeting (Berlin)
  https://thepickledfish.wordpress.com/2016/07/05/fluid-collections-workshop-spnhc2016/
  Presentations *Basic Collection techniques I & II*
- April 2017: working meeting, Natural History Museum (London)
- May 2018: Smithsonian Museum Meeting (Washington DC)

Introduction

- The Clothmakers Foundation Expert Workshop on Benchmark Standards for the Preservation of Wet Collections.
- Considerations and baseline guidelines for the collection, care and conservation of fluid collections



Baseline standards – Overview of Concept

The principle aims of the project are

to establish what we do and don’t know about fluid collections

to develop baseline best practices for their storage & management,

to develop an outline for a training syllabus for their care, and

to identify future research needs.

Ultimately these collections are our cultural heritage, not just a scientific resource.

Baseline standards – Overview of Concept

1. A baseline is the basic requirement to be met to maintain the collection in stable condition

Different possibilities for reaching the baseline (categories)

2. Achieving the baseline must be economically achievable for the institution

3. Achieving the baseline takes into account collection size, environmental settings, and frequency of collection use

Overview of recommended practice.

1. Fixation and preservation of specimens
2. Interactions of specimens and preserving fluids
3. Specimen containers
4. Storage environment
5. Review of factors that affect the long term usefulness of fluid preserved specimens
6. Sustainability and future research

It is unethical and unsustainable to preserve specimens in a way that makes them unusable for display, education or scientific investigation.

Basic considerations when collecting specimens

Fixation

→ What is the purpose of the specimen (research, anatomical preparation)?

→ What will it be used for later (reference material, display specimen)?

Specimen

→ Does the specimen require a specific fixation technique (vertebrate/invertebrate, plant/animal)?

Environmental considerations

→ Will environmental conditions influence fixation (marine vs freshwater; osmolarity)?

→ Does climate affect fixation technique (tropical vs artic temperatures)?

Location

→ Will the location influence the fixation process (transporting freshly fixed specimens on bumpy roads, fixation on board a ship)?

Field collecting – consider the challenges of the environmental conditions

Setting: Nile River, early afternoon, ca. 100 specimens, air temperature ~ 45 °C

What are your recommendations ?

Basic considerations when collecting specimens

Basic considerations when collecting specimens

Failure to fix specimens in a narcotised and relaxed condition often results in useless specimens.

→ Fix specimens **narcotised and free of pain** in a relaxed condition to ensure the best possible preparation results in a reasonable time span (in dependence of the respective organisms).

- ✓ Use a container of appropriate size for the organism
- ✓ Use an appropriate chemical to narcotise the target organisms
- ✓ Use an anesthetic concentration of appropriate strength (avoid overdosing)
- ✓ Consider metabolism rate of target species
- ✓ Consider external environmental factors (e.g., cooling in tropical climate, no direct sun exposure)

Factors that affect usefulness of fluid preserved specimens

- Narcotisation and euthanisation
- Length of time between death and fixation/preservation
- Quality of fixative and preservative solutions
- Rate of penetration of fixative/preservative
- Temperature of fixation/preservation
- Proportion of fluid volume to specimen (should be at least 7:3)

Fixation vs Preservation

→ **Fixation =** structural stabilisation (especially of lipids and proteins) by arresting and preventing post mortem changes

→ **Preservation =** keeping perishable materials for long time periods in stable and usable condition

Fixatives

Baseline standard: Effective fixation

- ✓ Fast diffusion and permeation in tissues (varies with ambient conditions)
- ✓ Rapid cessation of enzymatic activities (autolysis) with exposure to fixative
- ✓ Prevention of osmotic collapse of cells, organs and other components of the organism
- ✓ Minimal shrinkage or distortion of the specimen during fixation
- ✓ Protection from microbial activity
- ✓ Keep specimen stable until transfer to permanent preservative and proper collection storage environment

Fixation

Selecting a fixation technique:

→ What is the future use of the specimen (research, exhibition, teaching?)

→ How will the specimen be maintained (size, accessibility)

→ Research criteria (e.g., molecular or histological research processes)

→ Physical condition and osmotic pressure (e.g. marine vs. fresh water environment)

→ Environmental factors that influence fixation process

Fixation

Fixation

Too slow

Too weak

Fixation

Aldehyde fixation:

→ Aqueous formaldehyde = saturated solution of formaldehyde gas in water

- ✓ Concentration may be listed as 37% (w/w) or 40% (w/v)
- ✓ 8-13% methanol added to prevent polymerization of paraformaldehyde

→ Standard formalin fixative = 1 part formaldehyde + 9 parts water

→ Formalin must be neutral buffered (carbonate / phosphate buffer

- ✓ Phosphate buffers considered to be the most stable
- ✓ Some prefer calcium carbonate buffers

→ pH of unbuffered aqueous formaldehyde ≈ 2.5 – 3.5

→ pH of 1:9 aqueous formaldehyde and water ≈ 3.0 – 4.6 % formaldehyde in solution

→ < pH 6 formaldehyde rapidly forms formic acid

→ Mucus or integument structure may lower perfusion rate and fixation process

Fixation

Special case: Glutaraldehyde fixation (SEM)

→ 0.1 – 1.0 % glutaraldehyde and water solution

→ Lower perfusion rates, less penetration of tissues

→ Preferred for some small invertebrates (particularly for marine invertebrates)

Fixation

Alcohol as a “fixative”:

→ Works as fixative only at high concentrations

- ✓ Not a real fixative, but has certain fixative properties
- ✓ Fixative effects from dehydration of tissues (causes shrinkage)

Ethanol (pseudo) “fixation”

→ 95.6 % (highest concentration achieved by distillation)

→ Ethanol concentration > 95.6%mind is chemically dehydrated

→ Water displaced from tissues rapidly dilutes “fixative” alcohol (use 3:1 ratio of ethanol volume to tissue volume)

Fixation

Heavy Metal fixation

→ Osmium tetroxide, OsO_4

→ Used for transmission and scanning electron microscopy

→ Highly toxic

Preservation

Effective long-term preservation

- ✓ Prevent enzymatic deterioration (autolysis) and microbial attack
- ✓ Ameliorate morphological and molecular changes
- ✓ Maintain specimen in as natural a state as possible
- ✓ Give structural support to the specimen
- ✓ Create a stable microenvironment within the container

Preservatives

Storage media in wet collections include:

- Glycerine
- Aldehydes—formaldehyde, glutaraldehyde
- Alcohols—ethanol, isopropanol, glycerol
- Oils
- Aromatic solvents—turpentine, benzoates (for transparencies)
- Acids—preservatives or additives (e.g., acetic acid)
- Proprietary fixatives and preservatives
- DMDMH—dimethylodimethyl hydantoin (a formaldehyde releasing agent)
- Glycols—propylene glycol, ethylene glycol

Preservatives

Typical storage fluids

→ Denatured or undenatured ethanol (e.g., IMS/IDA)

- ✓ Ideal concentration 70 - 75 %
- ✓ 70 % and above is a strong biocide
- ✓ Below 50 % is ineffective as a biocide

Preservatives

Typical storage fluids

→ Aqueous formaldehyde

- ✓ Recommended concentration = 1:9 formaldehyde and water
- ✓ Decalcification of some tissues may begin at pH 6.4 or below
- ✓ Clearing of some tissues may begin at pH 7.0 and above
- ✓ Preferred pH for storage ~ 6.0
- ✓ Must use long-term neutral buffer

Preservatives

Typical storage fluids

→ Glycerine

- ✓ Use concentration 50 – 100 %
- ✓ Add menthol or thymol as biocide
- ✓ Extraction of water from tissues or absorption of relative humidity from air may dilute glycerine (use 3:1 glycerine to tissue volume ratio)

Buffers in fixing and preserving fluids

→ pH values < 6.5 cause decalcification and hardening of specimens

→ pH values > 7.0 leach proteins and lipids which clear soft tissues

✓ recommended pH: 7 +/- 2 (range 5 to 8)

→ Standard phosphate buffer system (Na_2HPO_4 and NaH_2PO_4)

✓ may precipitate out of solution when topping up or changing fluids

→ Calcium carbonate buffer

Collection Management

Topping up

→ Consider topping up strategy when the fluid level in the container has decreased

→ **Each container has its own storage micro environment**.

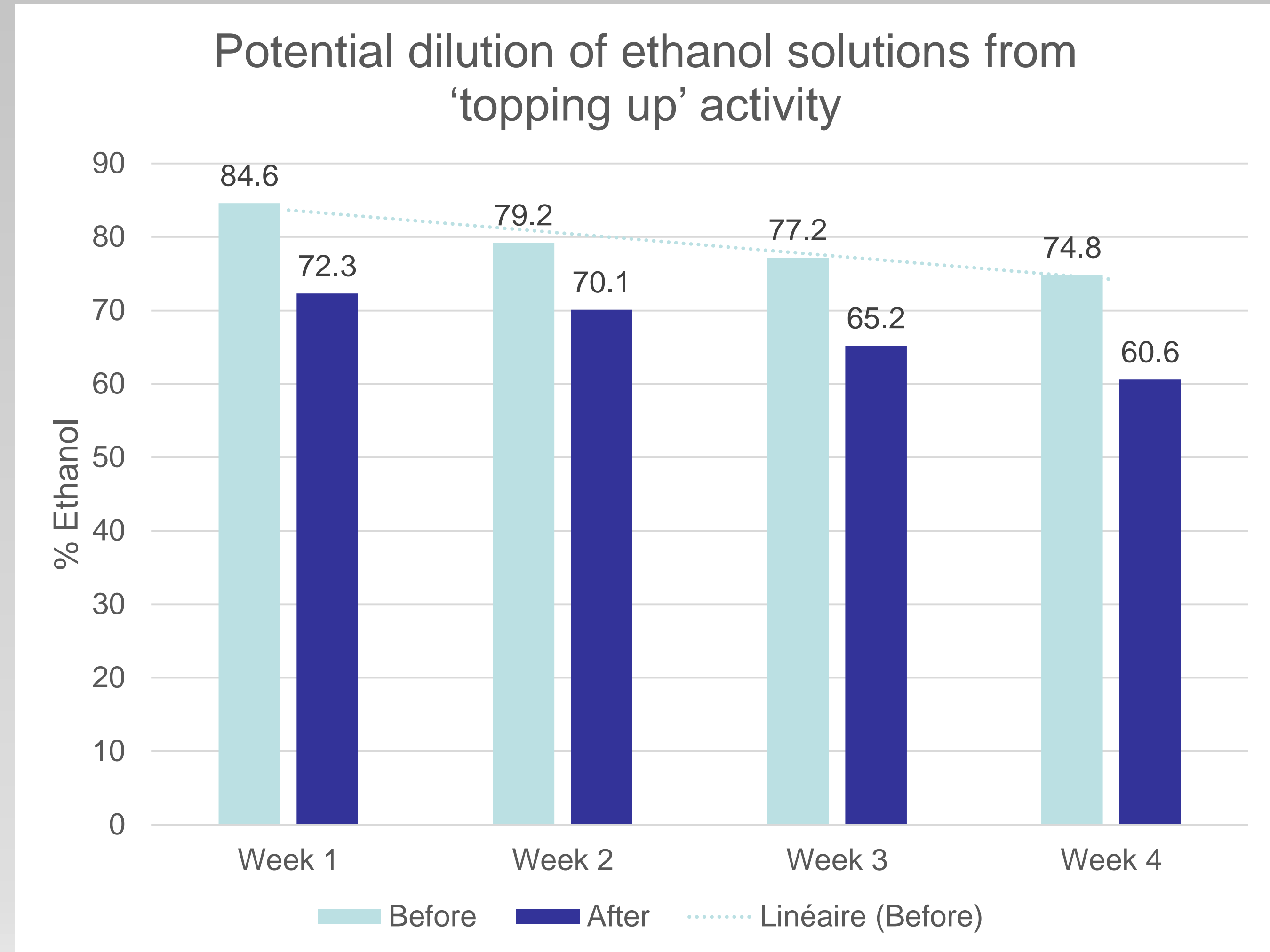
→ Preservative or fixative must be known or identified

- ✓ Do not change fluid and disrupt internal equilibrium randomly
- ✓ If fluid is unknown leave it alone or identify it

Collection Management

→ Determine the concentration of preservation fluid remaining in the container

- ✓ Adding 70 % to fluids with lower concentration will not reach target concentration
- ✓ Topping up delicate specimens with higher concentrations can cause osmotic problems
- ✓ Adding too much fresh alcohol might cause air bubbles to enter specimens



Collection Management

Should you change the fluid?

→ Ascertain that it is absolutely necessary to exchange the preservative fluid in a specimen container

→ Do not change storage fluids randomly

→ Changing fluids changes the equilibrium established between the fluid and the specimen.

Collection Management

Changing solutions

→ Precipitate accumulation on specimens and in the bottom of the container

→ Sudden cloudiness appears when topping up fluid is added

- ✓ *Mixing effects can identify potential denaturants in a solution such as petroleum ether (which requires fluid change)?*

→ Preservation fluid fails (e.g. hydrolysis of specimens)

→ Excessive acidification of the fluid from lipid oxidation or formic acid formation.

Interactions of specimens and preserving fluids

Influence of specimens on storage fluids

→ pH shifts induced from stored specimens

- ✓Acids may lower pH (e.g., formic acid dissolved from ants or wasps)
- ✓Bases may increase pH (e.g., calcium hydroxide released from crayfish)

→ Chemicals used to euthanise specimens can trigger secondary reactions

→ Lipids released from specimens can trigger secondary reactions

→ Residual fixatives dissolving out of specimens can trigger secondary reactions

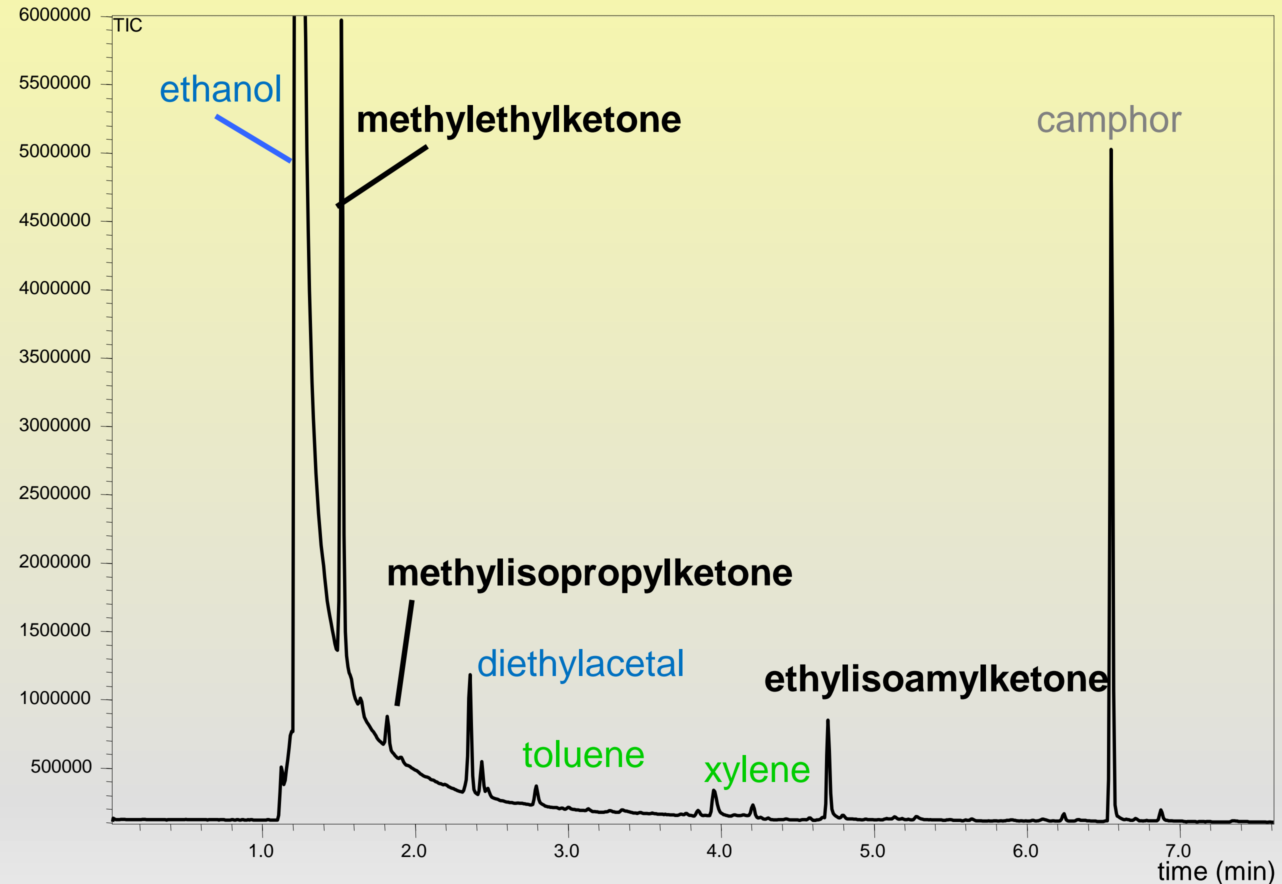
Conservation medium - KUR restauration project

Denaturing agents in ethanol:

Methylethylketone, **camphor**, **toluene + xylene** **(from benzine?)**

GC-MS chromatogram of one sample:

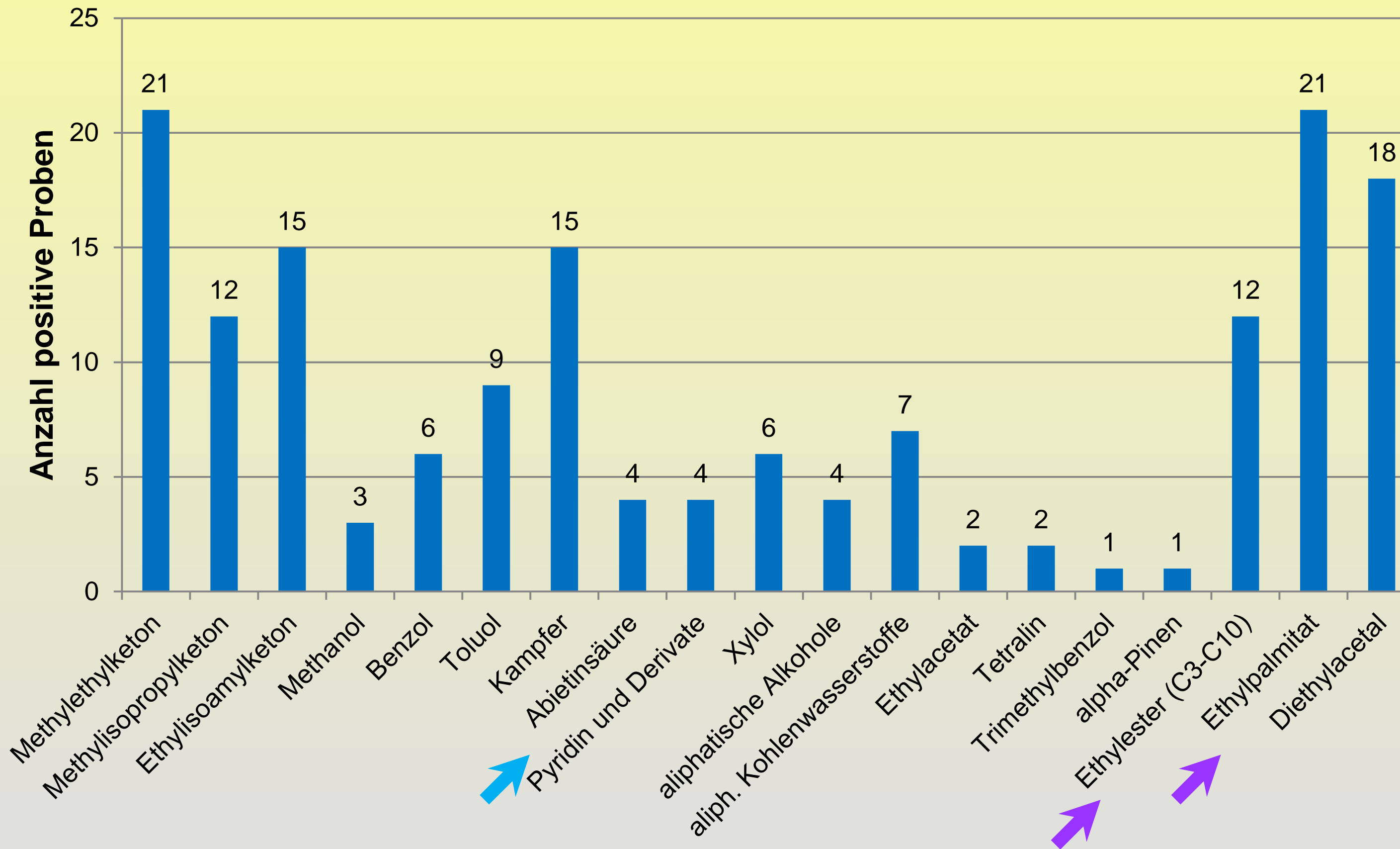
(25 vertebrate samples, old samples)



Source: J. Riedel, BAM, Workshop KUR 28.1.2011.

Conservation medium - KUR restauration project

Denaturing agents in ethanol:



Source: J. Riedel, BAM, Workshop KUR 28.1.2011.

Remarks: abietic acid – from denaturing of specimen or from seal colophonium?
ethylpalmitate, ethylester – from degradation of specimen,
no acetic acid (from ethanol).

Storage Containers

Storage containers should:

→ Be of appropriate size (height & width)

→ Support stable, upright positioning of the specimen inside the jar

→ Be durable over time and chemically inert

→ Provide a stable micro-environment

→ Allow external monitoring of specimens (without opening container)

→ Have a minimum volume not below 75 ml to reduce evaporation

Storage Containers

→ Soda-lime glass—matrix of SiO_4^- (73%), ions Al^{3+} (1%), Ca^{2+} (5%), Na^+ (17%)
  - ✓ ions dissolve, highly reactive surface layer

→ Borosilicate glass—
SiO_2 (81%), Al_2O_3 (2%), Na_2O (4%), B_2O_3 (13%)
  - ✓ ± chemically inert against leaching

→ PET containers (polyethylene terephthalate)—
colourless semi-crystalline resin
  - ✓ good gas, fair moisture barrier (hydroscopic, absorbs water)
  - ✓ good alcohol barrier (requires additional barrier treatment)
  - ⊖ UV light accelerates oxidation of surface (hydroxide & peroxide groups) in presence of air (oxygen) and humidity

→ Limited life of plastics:
PE (25 y), PP (10 y), PVC acrylic (< 3 y)

Glass Containers

Pros and Cons:

→ Glass with glass stopper
  - ✓ Chemically inert, easy access, low evaporation rate, expensive

→ Glass with glass lid and wire bail
  - ✓ Difficult to seal well, short gasket life

→ Glass with picein, beeswax, colophonium, or Alsirol seal
  - ✓ Good seal, but difficult to access specimens

→ Glass with threaded lid
  - ✓ Widely available, inexpensive, seal quality depends on thread style and lid material, easy access

→ Glass with plastic snap-on (torsion fit) closure
  - ⊝ Plastic loses elasticity, may crack

→ Glass with compressible stopper
  - ⊝ Stopper loses elasticity, high evaporation, contamination potential

Plastic Containers

Pros and Cons:

→ Most plastics susceptible to damage from denaturants (e.g., MEK)

→ PET (polyethylene terephthalate)

- ⊝ Untested, difficult to find good closure

→ Polycarbonate

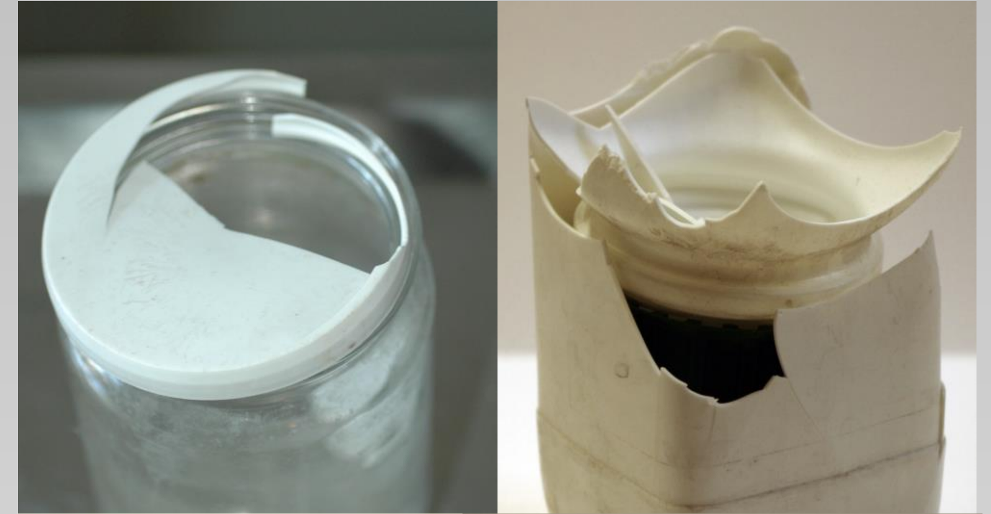
- ✓ Relatively inert material
- ⊝ difficult to find good closure

→ Acrylic

- ⊝ Preservatives pass through acrylic, seals break with flexing of container

→ High-density polyethylene HDPE)

- ⊝ Translucent (difficult to monitor specimens), susceptible to cracking after exposure to ultraviolet radiation



Containers for Large Specimens

Pros and Cons:

→ Ceramic crock with ceramic lid

- ⊝ Glaze breaks down over time, very difficult to seal

→ Stainless steel

- ✓ Quality depends on design of closure, quality of steel, and quality of welds
- ✓ V2A, Cr/Ni steel 18/10 recommended
- ✓ Ball valve inlets and outlets
- ⊝ Means to ground container against electrical charge
- ✓ Viton seal (Dupont)

Lids and closures

→ Minimize evaporative losses
→ Durable and chemically resistant
→ Ensure a stable micro-environment

Quality:

→ Glass closures
→ Recommend borosilicate glass, exact grounded joint
→ plan glass flange (e.g. historic battery jars)
⇒ requires additional sealant
Standardized lids (e.g. metal / plastic twist-off jars)
⇒ dependent on industrial demands and needs (e.g. food industry)
→ non-standardised lids (other metal / plastic lids)
⇒ might offer solutions for individual demands / can be customised

Sealants

→ increases performance of lids / closures

→ should be durable and (chemically) inert

→ should not include silicone or silicone based greases