❹ Result of the biomolecular preparation of a bone speciment

A rib with fibrous dysplasia of the bone was available as a surgical specimen.

In these benign tumour-like bone lesions, a detectable, specific biomolecular mutation in the GNAS1 gene is well-known, the detection of which can be important for diagnostic purposes.

After decalcification, a small amount of tissue was removed from a block and was processed in the biomolecular lab using DNA extraction and PCR.

Conditions of use and SONOCOOL settings

Type of sample:
Surgical specimen of a rib with a fibrous dysplasia of the bone

Device temperature: 24 °C

Ultrasonic output: 100 %

Decalcification duration: 48 h

Results and comments

It was possible to obtain a sufficient amount of tumour DNA of the required quality, and the sequencing of the PCR product yielded evidence of a mutation in this gene.

Exemplary formation of an SC 255 in connection with a pump for continuous replacement of the EDTA solution.

SONOCOOL® SC 255

Today, ultrasound is an integral part of both pathology and analysis laboratories where rapid diagnosis and a high degree of process effectiveness are required. SONOCOOL combines the process accelerating impact of ultrasound and simultaneous cooling of the sample.

During the histological analysis of samples, in oncology for example, bone decalcification is an important step as only decalcified samples can be suitably prepared for later analysis without contamination. The tedious process used to date can now be accelerated considerably using ultrasound.
The morphological structure of the sample, however, is preserved by the integrated cooling. The creation of a substandard sample, the diagnosis of which would be difficult or impossible, is avoided. The results are available in a shorter time.

Using SONOCOOL, the catalytic effect of ultrasound can now be used on heat sensitive samples in analysis laboratories. The cooling function means that exothermic reactions can be controlled and the processes are carried out faster and more effectively.

More information
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58020a e/2012-02

SONOCOOL®

Ultrasonic unit with cooling

for use in pathology
and analysis laboratories

BANDELIN
The Ultrasound Company

Four examples of application

❶ Examination of the decalcification process at variable ultrasonic output in a subjective comparison (cuttability test, microscopic analysis)

After 24 hours, each of the tissue samples was subjectively tested for cuttability by an MTA, using routine lab procedures. When cuttability was achieved, the samples were embedded in paraffin, H&E-stained slices were prepared, and their quality was evaluated microscopically.

Conditions of use and SONOCOOL settings

Type of sample:
Femoral head specimens, 4 mm-thick bone slices (include spongiosa, cortical bone, some joint cartilage)

Type of decalcification solution: buffered EDTA solution

Device temperature: 24 °C

Utrasonic output: 50 %, 75 % and 100 %

Decalcification duration: 17 h up to 89 h

Results and comments

All decalcified bone samples in this test underwent a complete demineralisation of the osseous tissue portions, with very good preservation of osseous and non-osseous tissue structures (spongiosa, cortical bone, marrow cavity tissue, cartilage, connective tissue).

Ultrasonic output	Block	Duration until the cuttability	
		hours	days
50 %	1	65 h 15 “	< 3
	5	65 h 15 “	< 3
	10	89 h 15 “	< 4
	11	65 h 15 “	< 3
	15	65 h 15 “	< 3
	16	65 h 15 “	< 3
75 %	2	79 h	> 3
	6	79 h	> 3
	7	79 h	> 3
	12	79 h	> 3
	13	48 h	2
100 %	3	48 h	2
	4	48 h	2
	8	17 h 30 “	< 1
	9	41 h 30 “	< 2
	14	41 h 30 “	< 2

Table to example 1: Duration until the cuttability of samples in dependence on the ultrasonic output.

❷ Examination of the decalcification process at variable ultrasonic output and variable decalcification solution, in an objective comparison (contact radiography)

After 24 hours, each of the tissue samples was examined by means of contact radiography, to be able to assess demineralisation using the X-ray image. After 48 hours of decalcification, the bone samples were embedded in paraffin, irrespective of their subjectively-evaluated condition. H&E-stained slices were prepared and their quality was evaluated microscopically.

Since, as expected, the compacta was not sufficiently decalcified in the gentle decalcification solution after 48 hours, it was removed prior to production of the paraffin blocks. Only the spongiosa was further processed histologically.

Conditions of use and SONOCOOL settings

Type of sample:
Tibial shaft specimens, 4mm-thick bone slices taken from an amputated lower leg (include 3–4 mm-thick compactas and spongiosas)

Type of decalcification solution: buffered EDTA solution and hydrochloric acid-containing medium

Device temperature: 24 °C

Ultrasonic output: 50 % and 100 %

Decalcification duration: 24 h and 48 h

Results and comments

With a hydrochloric acid-containing medium: extensive demineralisation of spongiosa and cortical bone after 24 hours, irrespective of ultrasonic output.

With the gentle medium: Decalcification of the spongiosa completed after 48 hours at 100 % ultrasonic output

Cuttability: free of problems for all prepared blocks

Histotechnical quality: very good, good structure preservation
No loss of quality at 100 % ultrasonic output

Note: As expected, the blocks decalcified with the hydrochloric acid-containing medium showed reduced colouration of the cell nuclei and of the bone matrix, constituting a reduction in quality for bone tumours or bone marrow.

❸ Results of decalcification for osteosarcoma

The osseous tissue blocks were produced in a well-established cutting process using a diamond cut-off grinding system.
Size: Lateral length 15 mm and thickness 3–4 mm
Three embedding capsules were processed in a glass jar.
A subjective evaluation of the cuttability was conducted.

Conditions of use and SONOCOOL settings

Type of sample:
Bone samples from surgical specimens of malignant bone-forming tumours in children and adolescents

Type of decalcification solution: buffered EDTA solution

Device temperature: 24 °C

Utrasonic output: 100 %

Decalcification duration: Varied between 3–5 days since the bone structure is very inhomogeneous in these samples (tumour spread and variably-pronounced tumour-specific bone formation).

Results and comments

Very good preservation of the tissue structure and of the tumour’s cell profile.

Stainability with routine colours and immunohistochemical reactivity is guaranteed.

Note: Bone biopsies for hematopathologic diagnosis (bone marrow, leukemia, or lymphoma diagnosis) were not used since material of this type is only available in small quantities and cannot be easily obtained.