

Analytical and clinical performance report:

ProbeAX Evolution tubes and SpinAX device for homogenization of tissue samples

Investigating laboratory:

Département ADMED Microbiologie
Dr. Antony Croxatto, director of microbiology department
Boucle de Cydalise 16
2300 La Chaux-de-Fonds

Table of contents

Introduction	2
Ethics and Anonymization	3
Methodology	3
Sample types	3
Sample preparation	3
Gram-staining and reading	5
Analysis	5
Results	6
Gram-staining	6
Solid and liquid culture	6
Conclusion	11

Introduction

Osteoarticular infections require accurate microbiological diagnostics with careful approaches for sampling, pre-analytical processing and diagnostic analysis. To increase the diagnostic accuracy, pre-processing steps such as homogenization or grinding of tissue samples are implemented. Different homogenization instruments are on the market today, for example Ultra-TurrAX® Tube Drive (IKA, Germany) and the associated ProbeAX tubes (Axon Lab, Switzerland) or the gentleMACS™ Tissue Dissociator (Miltenyi Biotec, Germany).

Axon Lab has developed a new homogenization instrument (SpinAX, Ref. AL0500) in conjunction with a new sampling and processing tube (ProbeAX Evolution, Ref. AL0510).

The ProbeAX Evolution is a single-use sampling tube containing 5 ml 0.85% NaCl. Inside the tube is a ceramic block with a rough surface and an aluminum grater on the inside of the tube-lid. The tube containing the sample is turned on its head and put into the SpinAX instrument. The instrument rotates at high speed (2200 rpm) in an oscillating motion, thus creating a grinding motion that grates the sample in between the ceramic block and the metal-grater. In the grating, tissue is broken down, biofilm is taken apart and bacteria or fungi are released into the physiological solution. An aliquot of this solution containing the released microorganisms can then subsequently be used for cultivation on agar plates, for enrichment broth or for molecular diagnosis of pathogenic organisms. The objective of this study is to demonstrate effectiveness of bacterial/fungal release from tissue samples using the SpinAX/ProbeAX Evolution homogenization system in comparison with Ultra-TurrAX/ProbeAX system as a reference method, which is currently used in our lab routine (Figure 1).



Figure 1. Left: SpinAX instrument with ProbeAX Evolution tube. Right: Ultra-TurrAX instrument with ProbeAX tube.

Ethics and Anonymization

No approval from the board of ethics is needed for this study, granted an effective anonymization of the patient sample.

For efficiency of the analytical procedure, the biological material for the research project is used at the same time as it is used for the routine diagnostic (one aliquot in routine, one aliquot for research). The results for the two methods then need to be compared. At the stage of inoculation of the sample onto agar plates and broth, the sample needs to be entirely anonymized with a random number (not linked to patient number). The information to be retained are: type of sample (bone, cartilage, tissue, etc.), link to other duplicate samples from the same patient. Time between arrival of sample in the laboratory and recording of result is <1 day. The date of the analysis will not be recorded. With this anonymization step, the patient should not be deduced anymore.

It is the responsibility of the technician, at the time of logging the result, to anonymize and give a random number to each sample.

Methodology

Sample types:

1. Soft tissue (fibrous, connective tissue, wound epithelium, etc.)
2. Medium to hard tissue (tendon, cartilage, bone)

If the sample is a mix of different tissue types, it should be categorized into the «harder» category.

Samples preparation

- Biopsy samples are dissected with surgical blade under laminar flow.
- Separate samples are splitted in two parts: one part for Ultra-TurrAX/ProbeAX homogenization with metal beads. The other part for SpinAX/ProbeAX Evolution homogenization with steel rasp (see Figure 2).
- The samples are placed into an Ultra-TurrAX and SpinAX tube, respectively, both containing 5 ml sterile 0.85% NaCl.
- The Ultra-TurrAX tube is homogenized with the Ultra-TurrAX® Tube Drive (Ref. 0003646000), according to the recommendation of the manufacturer of the device for 1 min. at speed level 6.
- The SpinAX tube (Ref. AL0510) is put into SpinAX (Ref. AL0500) and homogenized for 1 min. at 2200 rpm.
- From the homogenized solution, subsamples are taken to inoculate the following solid (4 quadrants methods) and liquid growth media – described in Table 1.

Figure 2

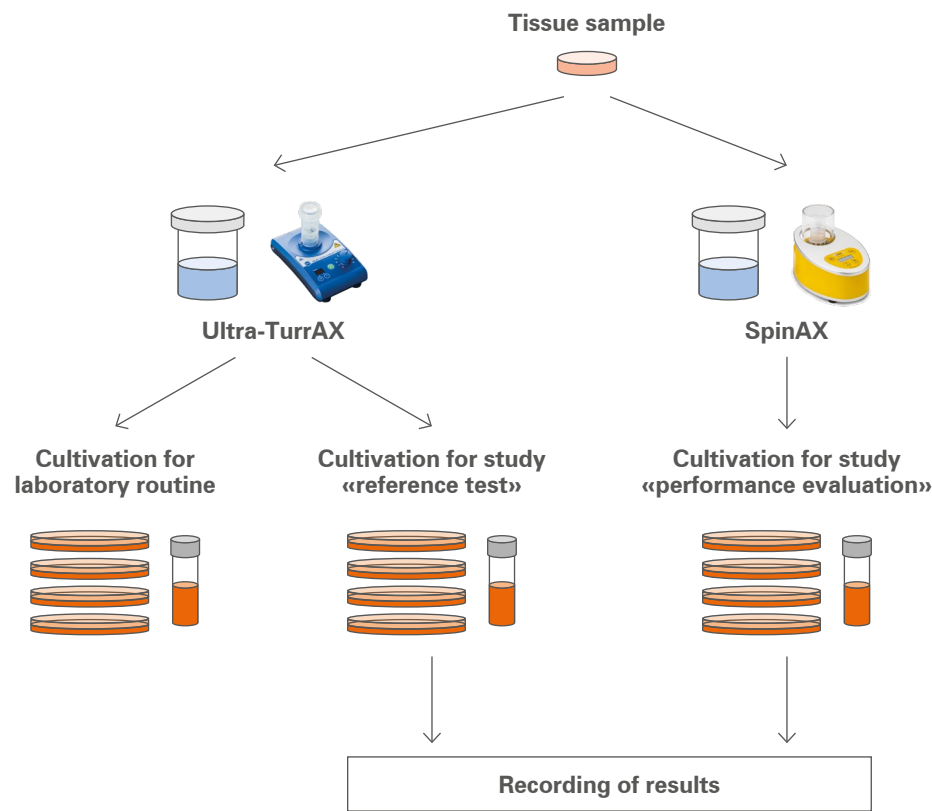


Table 1. Solid and liquid growth media, atmosphere, temperature and incubation times

Media types	Atmosphere	T°	Incubation times
Blood agar (GS)	5% CO ₂	35-37°C	24/48 h
Chocolate agar (CHOC)	5% CO ₂	35-37°C	24/48 h
Chromagar (ORI)	ambient atmosphere	35-37°C	24/48 h
Schaedler agar (SCH)	anaerobiosis (Scholzen)	35-37°C	48 h
Thioglycolate (THIO)	ambient atmosphere	35-37°C	5 d/14 d

- In addition a smear will be prepared for Gram-staining.
- After inoculation of the agar plates and broth, a liquid aliquot (500-1000 µl) from Ultra-TurrAX and SpinAX tubes will be frozen for long-term storage.
- Each agar plate will be incubated at 35-37°C, and carefully inspected at times T1 (18-24 hours), T2 (48 hours).
- The liquid media is incubated at 35-37°C and inspected for growth after T3 (5 days) and T4 (14 days).
- Growth/no-growth will be noted for each sample and agar type. When growth is observed, a semi-quantification will be reported (1+ to 4+) and an identification will be done by MALDI-TOF for each microbial morphology.
- Images are taken of each plate with bacterial growth.

Table 2. Categorization of semi-quantification results from 0 to 4 according to microbial growth observed in the different inoculated quadrants.

Growth	Semi-Quantification
4 th quadrant	4
3 rd quadrant	3
2 nd quadrant	2
1 st quadrant/Few CFUs	1
no growth	0

Gram staining and reading

- Slides will be Gram-stained with the Aerospray® Gram-slide stainer, ELITech France.
- The slides will be read by microscopy as described in our laboratory SOP. The following biological material will be reported (Leucocytes, Erythrocytes, Epithelial cells, Gram positive and Gram negative bacteria).

Table 3. Categorization of results according to quantity of cells or bacteria per microscopy field. Cells: epithelial cells, erythrocytes or leucocytes. Bacteria: Gram positive or Gram negative bacteria.

Cells/microscopy field (100x)	Bacteria/microscopy field (100x)	Quantification
>25	>25	4
6-25	6-25	3
2-5	2-5	2
1	1	1
no cells	no cells	0

Analysis

- The results will be recorded in a database.
- The SpinAX results will be compared to the Ultra-TurrAX results. Similar and discordant results will be measured between the 2 different methods. A detailed description of the discordances will be presented.

Results

Gram-staining

The global results are presented in Table 4.

Table 4. Comparison of cell count after Gram staining. Percent of total (absolute numbers).

Gram staining	Cell count with both methods	Cell count with the SpinAX only	Cell count with the Ultra-TurrAX only
GPC	52% (13/25)	36% (9/25)	11% (3/25)
GNB	45% (5/11)	45% (5/11)	9% (1/11)
Erythrocytes	85% (73/86)	12% (10/86)	3% (3/86)
Leucocytes	74% (61/82)	18% (15/82)	7% (6/82)
Epithelial cells	33% (2/6)	50% (3/6)	17% (1/6)
Total	73% (154/210)	20% (42/210)	7% (14/210)

GPC: Gram positive Cocci, GNB: Gram negative bacilli

Microorganisms and cells not seen by microscopy with Ultra-TurrAX or the SpinAX but observed with the other method are listed in Table 5.

Table 5. Microorganisms and cells not observed by microscopy (Gram staining with the SpinAX or the Ultra-TurrAX).

	SpinAX (S/MH)	Ultra-TurrAX (S/MH)	Total (S/MH)
GPC	1 (0/1)	5 (3/2)	6 (3/3)
GNB	0 (0/0)	2 (1/1)	2 (1/1)
Erythrocytes	0 (0/0)	0 (0/0)	0 (0/0)
Leucocytes	0 (0/0)	2 (2/0)	2 (2/0)
Epithelial cells	0 (0/0)	1 (1/0)	1 (1/0)
Total	1 (0/1)	10 (7/3)	11 (7/4)

GPC: Gram positive Cocci, GNB: Gram negative bacilli, S: Soft biopsy, MH: Medium to hard biopsy

Solid and liquid culture

A total of 96 clinical samples biopsies, 31 medium to hard and 65 soft, from 58 patients have been analyzed by culture following Ultra-TurrAX and SpinAX homogenization (Table 6).

Table 6. Number of specimen and patients included in the study

	Biopsy		
	Soft	Medium to hard	Total
Patients	34	24	58
Samples	65	31	96

Samples

Among the 96 clinical samples biopsies, 42 samples (43.8%) from 26 patients (44.8%) showed bacterial growth on at least one of the four agar (chocolate agar, chromogenic agar, blood agar and Schaedler agar) with at least one of the two methods (Table 7).

Table 7. Overall results of the growth observed on the different media following SpinAX or Ultra-TurrAX homogenization.

Item	SpinAX N° (%)	Ultra-TurrAX N° (%)	Total N° (%)
Samples			
Positive samples	42 (43.8%)	39 (40.6%)	96 (100%)
Negative samples	54 (56.3%)	57 (59.4%)	96 (100%)
False negative samples	0 (0%)	3 (3.1%)	96 (100%)
Global growth			
Similar Growth	84 (89.4%)	84 (89.4%)	94 (100%)
Better growth	4 (4.3%)	6 (6.4%)	94 (100%)
Clinical isolates			
Recovered clinical isolates	84 (89.4%)	83 (88.3%)	94 (100%)
Not recovered clinical isolates	10 (10.6%)	11 (11.7%)	94 (100%)
<i>Anaerococcus vaginalis</i>	1 (1.1%)	0 (0%)	94 (100%)
<i>Candida albicans</i>	0 (0%)	1 (1.1%)	94 (100%)
<i>Corynebacterium amycolatum</i>	1 (1.1%)	0 (0%)	94 (100%)
<i>Corynebacterium resistens</i>	1 (1.1%)	0 (0%)	94 (100%)
<i>Enterococcus faecalis</i>	2 (2.1%)	0 (0%)	94 (100%)
<i>Finegoldia magna</i>	1 (1.1%)	0 (0%)	94 (100%)
<i>Lactobacillus paracasei</i>	0 (0%)	1 (1.1%)	94 (100%)
<i>Moraxella osloensis</i>	1 (1.1%)	0 (0%)	94 (100%)
<i>Morganella morganii</i>	0 (0%)	1 (1.1%)	94 (100%)
<i>Peptoniphilus harei</i>	0 (0%)	2 (2.1%)	94 (100%)
<i>Porphyromonas spp</i>	1 (1.1%)	0 (0%)	94 (100%)
<i>Pseudomonas aeruginosa</i>	1 (1.1%)	0 (0%)	94 (100%)
<i>Streptococcus anginosus</i>	0 (0%)	1 (1.1%)	94 (100%)
<i>Staphylococcus aureus</i>	0 (0%)	4 (4.3%)	94 (100%)
<i>Staphylococcus epidermidis</i>	1 (1.1%)	1 (1.1%)	94 (100%)

* Similar growth is defined as a similar growth categorization or a growth categorization difference of 1 (≤ 1). Better growth is defined when there is a growth categorization superior to 1 (> 1) or when there is no growth (categorization of 0) with one method and growth with the other method (≥ 1).

False negative samples

Three false negative samples were observed with Ultra-TurrAX (Table 7).

- Two specimen of soft tissue from one patient exhibited a positive result with the SpinAX with a few colonies of *Staphylococcus aureus* and a false negative result for the Ultra-TurrAX. However, the *Staphylococcus aureus* strain was recovered from the Thioglycolate enrichment broth in one of the two specimen of the patient.
- A single specimen from one patient exhibited a positive result with the SpinAX with a few colonies of *Staphylococcus aureus* and a false negative result for the Ultra-TurrAX. No *Staphylococcus aureus* strain was recovered from the Thioglycolate enrichment broth in this specimen for the Ultra-TurrAX.

Global growth

Out of the 94 clinical isolates, 6 (6.4%) exhibited a significant better growth (categorization difference >1) following Ultra-TurrAX homogenization of 4 different specimen (3 medium to hard and 1 soft tissues) whereas 4 (4.3%) showed a better growth following SpinAX homogenization of 4 different specimen (2 medium to hard and 2 soft tissues). Thus, 84 (89.4%) clinical isolates showed a similar growth (growth categorization ≤ 1) following Ultra-TurrAX or SpinAX homogenization (Table 7).

Clinical isolates

Out of the 42 positive samples, 94 bacterial isolates belonging to 41 bacterial species were recovered on a least one of the four inoculated agar media (Table 7 and Table 8).

Table 8. Microorganisms recovered by culture on the different media following SpinAX or Ultra-TurrAX homogenization.

	Ultra-TurrAX	Neg	Pos	Pos	Total
	SpinAX	Pos	Neg	Pos	
Microorganisms					
Actinomyces europaeus				1	1
Anaerococcus vaginalis			1		1
Bacteroides fragilis				1	1
Bacteroides thetaiotaomicron				2	2
Bilophila wadsworthia				1	1
Candida albicans		1		1	2
Citrobacter freundii complex				1	1
Citrobacter koseri				1	1
Clostridium ramosum				1	1
Corynebacterium amycolatum			1		1
Corynebacterium resistens			1		1
Corynebacterium striatum				1	1
Enterobacter cloacae complex				4	4
Enterococcus faecalis			2	3	5
Escherishia coli col				1	1
Finegoldia magna			1	1	2
Fusobacterium nucleatum				1	1
Haemophilus parainfluenzae				1	1
Klebsiella oxytoca				1	1
Klebsiella variicola				1	1
Lactobacillus paracasei		1			1
Moraxella osloensis			1		1
Morganella morganii		1		2	3
Parvimonas micra				1	1
Peptoniphilus grossensis				1	1
Peptoniphilus harei		2			2
Peptoniphilus ivorii				1	1
Peptoniphilus spp				1	1
Porphyromonas sp			1	1	2
Proteus mirabilis				5	5
Pseudomonas aeruginosa			1	3	4
Schaalia radingae				1	1
Staphylococcus aureus		4		19	23
Staphylococcus epidermidis			1	1	2
Staphylococcus epidermidis (large)				3	3
Staphylococcus epidermidis (small)		1		2	3
Streptococcus agalactiae				1	1
Streptococcus anginosus		1		1	2
Streptococcus milleri group				1	1
Streptococcus pyogenes				5	5
Veillonella parvula				1	1
Total		11	10	73	94

Eleven bacterial isolates (11.7%) from 9 different specimen and 7 different patients were not recovered following Ultra-TurrAX homogenization (Table 7, 8 and 9).

Table 9: Clinical isolates not recovered following Ultra-TurrAX homogenization.

Patient-specimen	Soft/Hard-medium	Microorganisms	Incubation time	Semi-quantification of the SpinAX	Biologically acceptable (0/1)	Ano. Spe
21-1	Soft tissue	<i>Candida albicans</i>	48 h	1	Yes	No
21-1	Soft tissue	<i>Lactobacillus paracasei</i>	48 h	1	Yes	No
13-1	Soft tissue	<i>Morganella morganii</i>	48 h	3	No	No
18-1	Medium to hard tissue	<i>Peptoniphilus harei</i>	48 h	3	No	No
18-2	Medium to hard tissue	<i>Peptoniphilus harei</i>	48 h	3	No	No
4-1	Medium to hard tissue	<i>Staphylococcus aureus</i>	48 h	1	Yes	No
12-1	Soft tissue	<i>Staphylococcus aureus</i>	48 h	1	Yes	No
23-1	Soft tissue	<i>Staphylococcus aureus</i>	48 h	1	Yes	No
23-2	Soft tissue	<i>Staphylococcus aureus</i>	48 h	1	Yes	No
7-3	Soft tissue	<i>Staphylococcus epidermidis</i>	48 h	1	Yes	Yes
21-1	Soft tissue	<i>Streptococcus anginosus</i>	48 h	1	Yes	No

Biologically acceptable (0/1): Considered as «Yes» if there is poor growth (few colonies, first quadrant = growth score of 1) with the other method. Ano. Spe: Found in another specimen of the same patient

Ten bacterial isolates (10.6%) from 9 different specimen and 8 different patients were not recovered following SpinAX homogenization (Table 7, 8 and 10).

Table 10: Clinical isolated not recovered following SpinAX homogenization

Patient-specimen	Soft/Hard-medium	Microorganisms	Incubation time	Semi-quantification of the Ultra-TurrAX	Biologically acceptable (0/1)	Ano. Spe
1-1	Soft tissue	<i>Staphylococcus epidermidis</i>	48 h	2	No	No
2-1	Soft tissue	<i>Pseudomonas aeruginosa</i>	48 h	1	Yes	No
4-1	Medium to hard tissue	<i>Corynebacterium amycolatum</i>	48 h	1	Yes	No
9-1	Medium to hard tissue	<i>Anaerococcus vaginalis</i>	48 h	3	No	No
9-1	Medium to hard tissue	<i>Finegoldia magna</i>	48 h	3	No	No
18-1	Medium to hard tissue	<i>Porphyromonas sp</i>	48 h	3	No	Yes
21-1	Soft tissue	<i>Moraxella osloensis</i>	48 h	1	Yes	No
22-1	Medium to hard tissue	<i>Corynebacterium resistens</i>	48 h	2	No	No
25-1	Soft tissue	<i>Enterococcus faecalis</i>	48 h	1	Yes	No
25-2	Soft tissue	<i>Enterococcus faecalis</i>	48 h	1	Yes	No

Biologically acceptable (0/1): Considered as «Yes» if there is poor growth (few colonies, first quadrant = growth score of 1) with the other method. Ano. Spe: Found in another specimen of the same patient.

As shown in tables 9 and 10, clinical isolates not recovered following SpinAX or Ultra-TurrAX homogenization but isolated with a semi-quantification score > 1 with the other method were mainly observed with medium to hard tissues (6 out of 7 samples).

Conclusion

The SpinAX device in conjunction with the ProbeAX Evolution tube has an homogenization efficiency equal to Ultra-TurrAX device in conjunction with the ProbeAX tubes with 89.4% of the specimen showing similar bacterial growth.

Most of the discrepancies are very likely explained by biological variation of the microbial load that is not uniformly distributed in a tissue sample. Indeed, as the tissue samples were split into 2 parts for this performance evaluation, discrepancies can be expected with different growth recovery or absence of growth following one of the 2 methods when there is a weak bacterial load that is not systematically recovered by culture (Model of Poisson, low probability). In addition, discrepancies with a high bacterial load could be expected when the microorganisms are not equally distributed in the specimen. Indeed, the most significant differences (discrepancies with semi-quantitative differences >1) were mainly observed with medium to hard tissues that are difficult to process and to split into two equal parts (Table 9 and 10).

The SpinAX seems to allow a better detection of microorganisms and host cells by Gram staining microscopy (Table 4 and 5). Ultra-TurrAX microorganisms or host cells were not detected in 10 samples with: GPC not observed in 5 samples, GNB in 2 samples, Leucocytes in 2 samples and Epithelial cells in 1 sample, whereas in only one sample after SpinAX homogenization. However, these differences of Gram staining microscopy could also be explained by the lack of reproducibility of this analysis as showed in an Internal quality assurance investigation performed in diagnostic microbiology (see Scherz

et al, PLOS One, 2017, <https://doi.org/10.1371/journal.pone.0187263>). Indeed, in this study, 39.5% of major discrepancies of the Gram staining preparation and reading were observed from the same sample processed twice by the diagnostic laboratory.

Overall, the SpinAX homogenization is thus providing an overall similar performance than Ultra-TurrAX with a possible better homogenization and/or ultra-structure preservation for Gram staining microscopy. We have also observed macroscopically a better homogenization with the SpinAX than Ultra-TurrAX with the absence of aggregates after homogenization. The pipetting of the SpinAX supernatant was thus also easier to perform. Finally, the reduced noise produced during the SpinAX grinding and homogenizing compared to Ultra-TurrAX was really appreciated by the laboratory staff.

Study sponsor:

Axon Lab AG
Täferstrasse 15
5405 Baden-Dättwil