# Adjudication report

## Analysis of possible mix-up of slides in experiment 9

### Background

There is a suspicion that two patch groups have been mixed-up during biopsy in experiment 9 (6 weeks survival term) of the biocompatibility study of a novel lung sealant and consequently received the wrong naming on the cassettes.

Experiment design:

- Four sheep at each survival term: 5 days, 2 weeks and 6 weeks.
- Three groups per sheep: GATT-Patch (G), TachoSil (T) and untreated control (C).

### Initial assessment based on available data

***Findings***

1. Slides that are named E9-G1 and E9-G2 show an aspect of remaining fibrinoid patch material that does not correspond to the remaining patch material in other GATT-Patch slides on 6 weeks. *See evidence #1.*
2. The aspect of this remaining patch material shows more resemblance to another sample of the TachoSil group at 6 weeks: E11-T1 and E11-T2. *See evidence #2.*
3. Other slices of the GATT-Patch group show no more remaining patch material at 6 weeks. At two weeks, the GATT-Patch looks distinctly different on histology compared to the sections E9-G1/2 and E11-T1/2. *See evidence #3.*
4. Analysis of the cassettes and slices has shown that a mix-up could not have occurred in the naming process.
5. In experiment 9, according to the forms and confirmed on the implantation photos: TachoSil was applied to the right lower lobe, ventral part (diaphragmatic). *See evidence #4.*
6. GATT-Patch was applied to the right lower lobe, dorsal part. In the photo we can see the implantation process during which the lung is lifted using two gauzes in clamps. *See evidence #5.*
7. In experiment 9, pictures were marked with a paper that denoted T (for TachoSil) or G (for GATT-Patch).
8. In the pictures at obduction, the G-form is put next to a scar on the right lower lobe, ventral segment. *See evidence #6.*
9. It is not as clear from the photos what the location is, but the T-form is evidently not the same scar as seen in the ventral segment. *See evidence #7.*
10. The control lesion C-form is put next to a lesion on the right upper lobe. *See evidence #8.*

***Reasoning / deduction***

1. Based on findings 1-3, the question arises whether the GATT-Patch and TachoSil samples have been mixed up in E9.
2. Based on finding 4, if there is a mix-up, this must have occurred during the obduction, putting a tissue sample in the wrong cassette. In experiment 9 the sacrifice term was 6 weeks, and therefore, both patches are not macroscopically distinguishable anymore, making a mix-up of biopsies in the named cassettes possible. The only way to correctly identify lesions, is to take a biopsy of the right location on the lobe, as has been noted in the forms during index surgery.
3. Based on findings 5-10, we can synthesize that:
    a. The NHS-POx patch was applied to the right lower lobe (dorsal segment).
    b. At obduction the G-form (for NHS-POx) is put next to the right lower lobe (ventral segment).
    c. The fibrin patch is applied to the right lower lobe (ventral segment) during index surgery.
    d. The C-form is put next to a lesion on the right upper lobe, which is consistent with the location described on the forms of the original implantation surgery.
4. Therefore, based on the converging evidence of the histological aspects (finding 1-2) and the mix-up of forms at obduction (finding 5-10), we can deduce that a mix up has likely occurred while samples the tissues for histology at obduction between the NHS-POx samples (E9-G1/G2) and fibrin patch samples (E9-T1/T2) but not the control samples (E9-C3).

## Additional independent analysis to support the suspicion

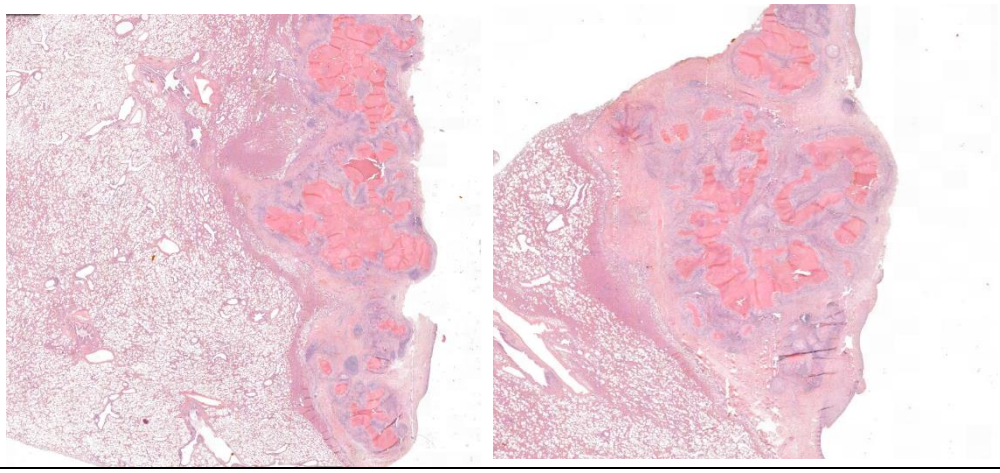
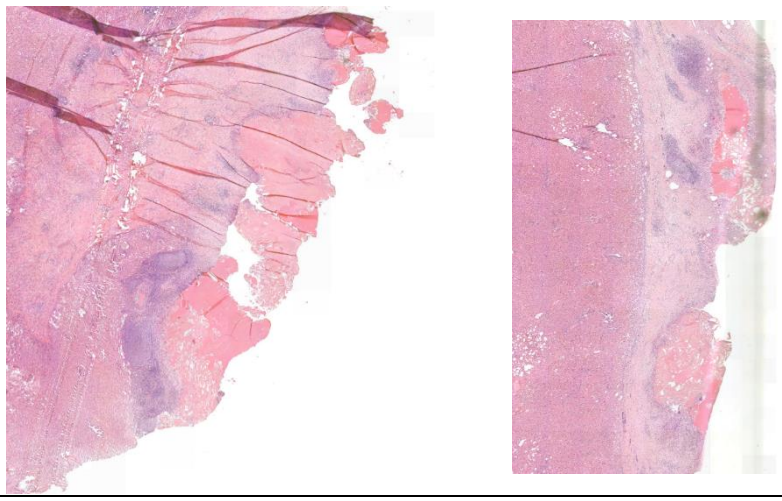
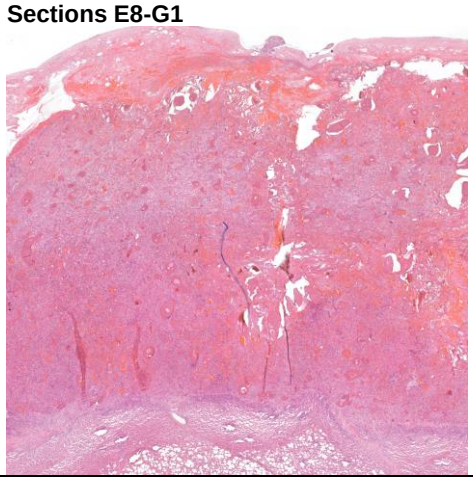
**Method:**

- Additional staining was obtained using ‘Elastine volgens Masson’ (EvM).
- As additional analysis, a blinded grouping task has been performed of various sections of patch material based on Hemotoxylin-Eosin (HE) and EvM staining by an independent pathologist, using baseline example slides of patch material groups.

**Findings**

- EvM staining also indicates that the patch remnant of E9-G1/G2 shows more resemblance to TachoSil than GATT-Patch. *See evidence #9.*
- The findings of the independent analysis also support the notion that the remannt material in E9-G1/G2 is TachoSil instead of GATT-Patch. *See supplemental evidence.*

# Evidence

| # | Evidence |
|---|---|
| 1 | **Sections E9-G1/G2**<br>  |
| 2 | **Sections E11-T1/T2**<br>  |
| 3 | **Sections E8-G1**<br>  |

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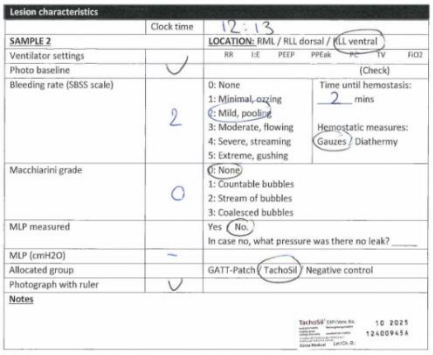
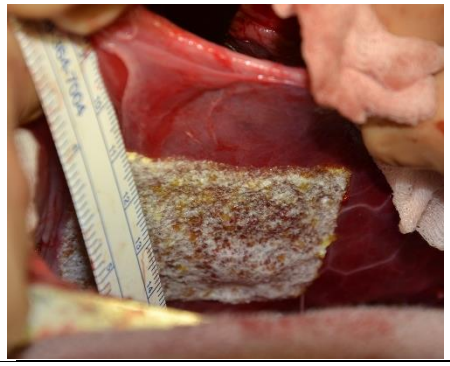
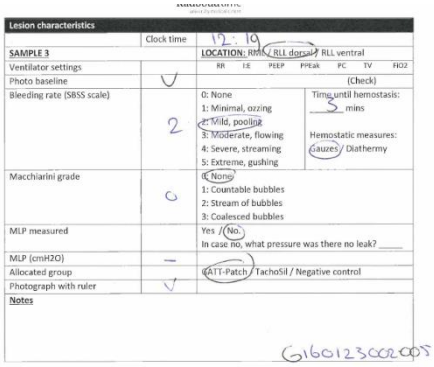
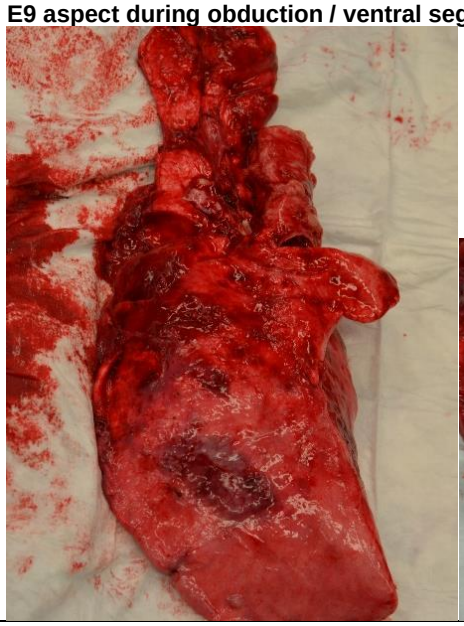
| 4 | **Form E9 / TachoSil implantation photo**<br>  <br>Lesion characteristics<br>Clock time: 12:13<br>SAMPLE 2 — LOCATION: RML / RLL dorsal / RLL ventral<br>Ventilator settings: RR I:E PEEP PPeak PC TV FiO2<br>Photo baseline: ✓ (Check)<br>Bleeding rate (SBSS scale): 2 — 0: None; 1: Minimal, oozing; 2: Mild, pooling; 3: Moderate, flowing; 4: Severe, streaming; 5: Extreme, gushing<br>Time until hemostasis: 2 mins<br>Hemostatic measures: Gauzes / Diathermy<br>Macchiarini grade: 0 — 0: None; 1: Countable bubbles; 2: Stream of bubbles; 3: Coalesced bubbles<br>MLP measured: Yes / No. In case no, what pressure was there no leak? ____<br>MLP (cmH2O): –<br>Allocated group: GATT-Patch / TachoSil / Negative control<br>Photograph with ruler: ✓<br>Notes<br>10 2025<br>124009458<br>  |
| 5 | **Form E9 / GATT-Patch implantation photo**<br>  <br>Lesion characteristics<br>Clock time: 12:10<br>SAMPLE 3 — LOCATION: RML / RLL dorsal / RLL ventral<br>Ventilator settings: RR I:E PEEP PPeak PC TV FiO2<br>Photo baseline: ✓ (Check)<br>Bleeding rate (SBSS scale): 2 — 0: None; 1: Minimal, oozing; 2: Mild, pooling; 3: Moderate, flowing; 4: Severe, streaming; 5: Extreme, gushing<br>Time until hemostasis: 5 mins<br>Hemostatic measures: Gauzes / Diathermy<br>Macchiarini grade: 0 — 0: None; 1: Countable bubbles; 2: Stream of bubbles; 3: Coalesced bubbles<br>MLP measured: Yes / No. In case no, what pressure was there no leak? ____<br>MLP (cmH2O): –<br>Allocated group: GATT-Patch / TachoSil / Negative control<br>Photograph with ruler: ✓<br>Notes<br>G160123002005<br>  |
| 6 | **E9 aspect during obduction / ventral segment**<br>  <br>  <br> |

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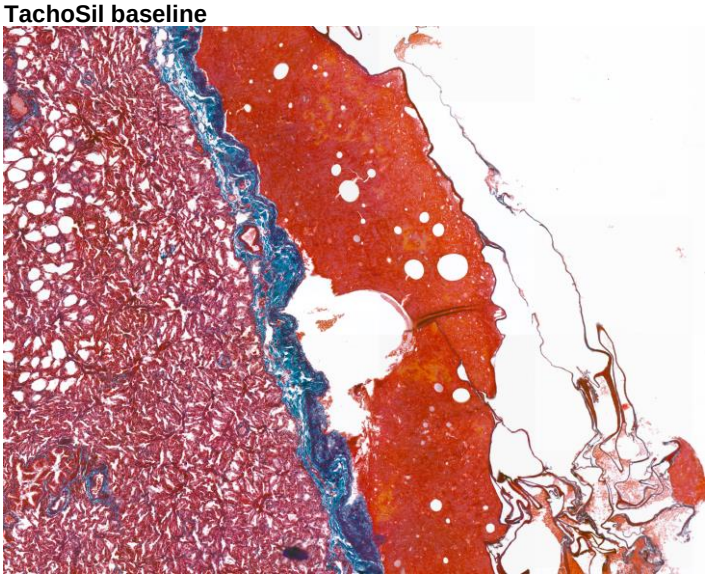
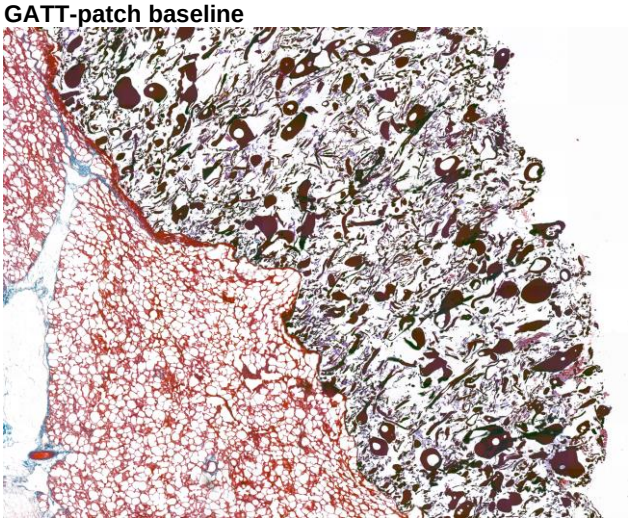
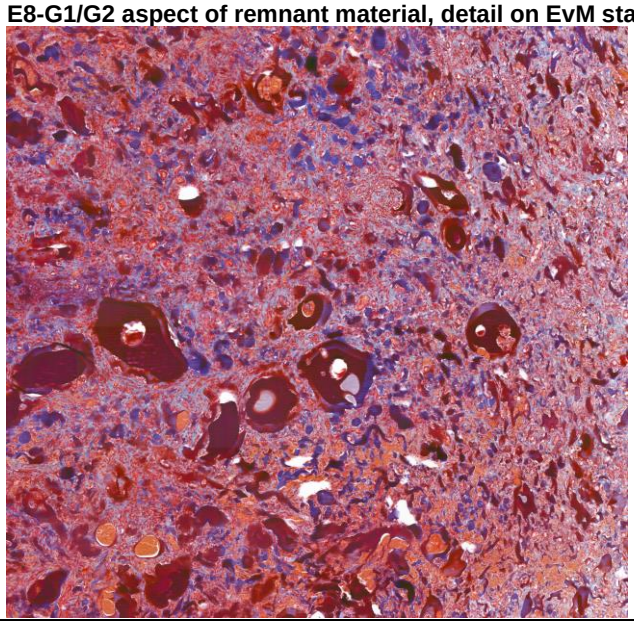
|---|---|
| 7 | **E9 aspect during obduction / dorsal segment**<br>  <br>  |
| 8 | **E9 aspect during obduction / control lesion**<br>  |
| 9 | **TachoSil baseline**<br>  |

**GATT-patch baseline**



**E9-G1/G2 aspect of remnant material, detail on EvM stain**

**E8-G1/G2 aspect of remnant material, detail on EvM stain**



## Conclusion

There is strong converging evidence based on macroscopic images, notes taken during the experiment and independent and blinded histological analysis (based on protocolar and additional staining) that the samples E9-G1/G2 and E9-T1/T2 have been mixed up during biopsy.

# Supplemental evidence: blinded and independent histological assessment

## Aim:

- To confirm that the histological aspects of the patch remnants are distinctly different.
- Substantiate the correction of the histological mix-up.

## Method:

- Assessment by independent pathologist.
- Hemotoxylin-Eosin (HE) and 'Elastine volgens Masson' (EvM) staining.
- Two baseline slides are shown to the pathologist, corresponding to the patches at baseline.
- Six other slides are shown per staining method, at 2 and 6 weeks.
- Slides are randomized and blinded.
- The task is to organize the randomized slides according to the suspected patch group, or 'no remnant on pleural surface'.

## Results:

- After unblinding the grouped sections, the results show that the section E9-G1 was grouped in the TachoSil group instead of the GATT-Patch group on both HE and EvM staining.

### Assessment based on HE-staining:

| **Group 1: TachoSil** | | **Group 2: GATT-Patch** | | **No remnant on pleural surface** | |
|---|---|---|---|---|---|
| Code | Key | Code | Key | Code | Key |
| 1 | **E9-G1** | 4 | E8-G1 | 3 | E11-G1 |
| 2 | E8-T2 | | | 5 | E9-T1 |
| 6 | E11-T1 | | | | |

### Assessment based on EvM-staining:

| **Group 1: TachoSil** | | **Group 2: GATT-Patch** | | **No remnant on pleural surface** | |
|---|---|---|---|---|---|
| Code | Key | Code | Key | Code | Key |
| 2 | E8-T2 | 1 | E8-G1 | 3 | E11-G1 |
| 4 | **E9-G1** | | | 5 | E9-T1 |
| 6 | E11-T1 | | | | |