

A combination method for Detection of Animal Proteins

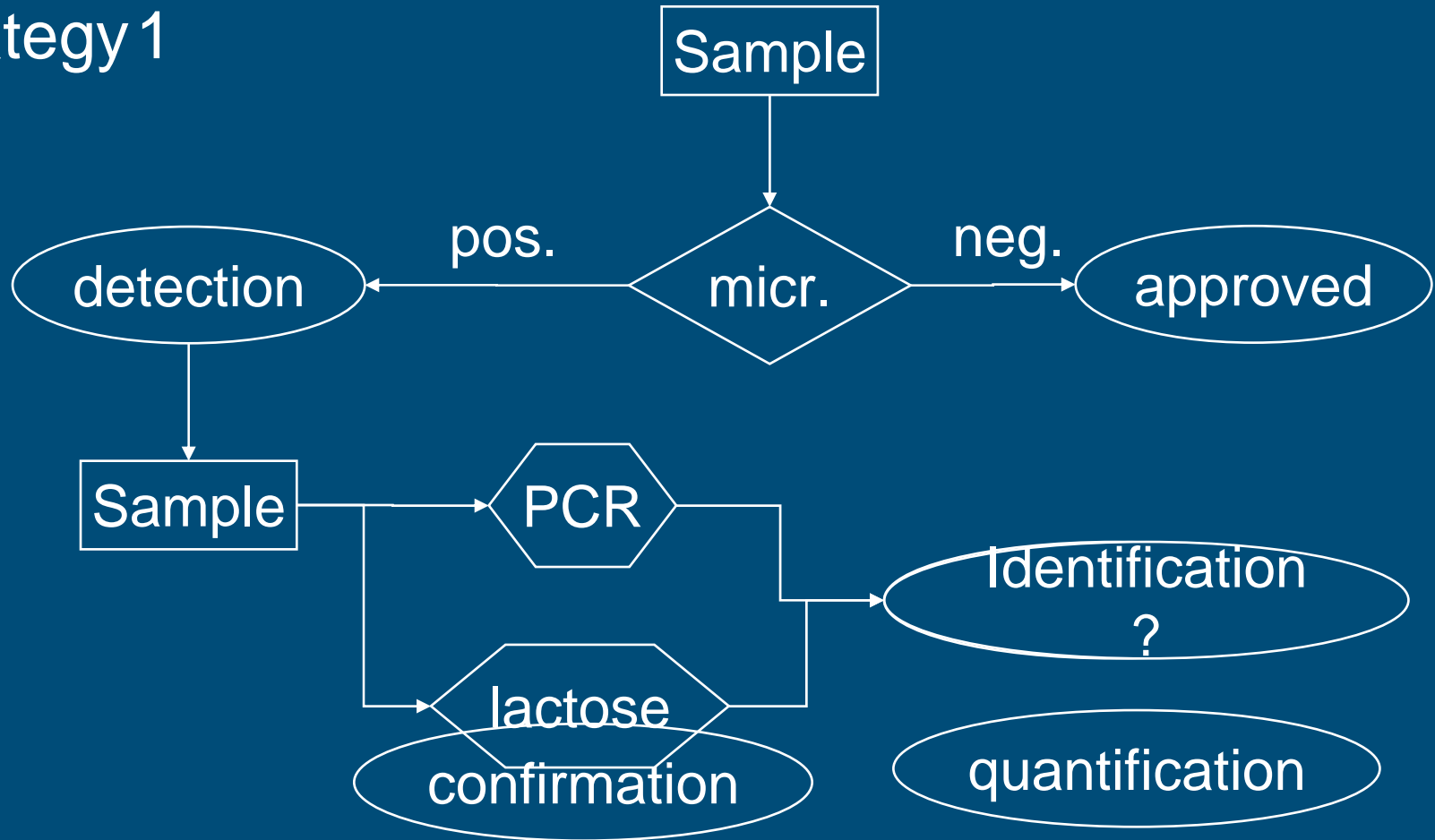
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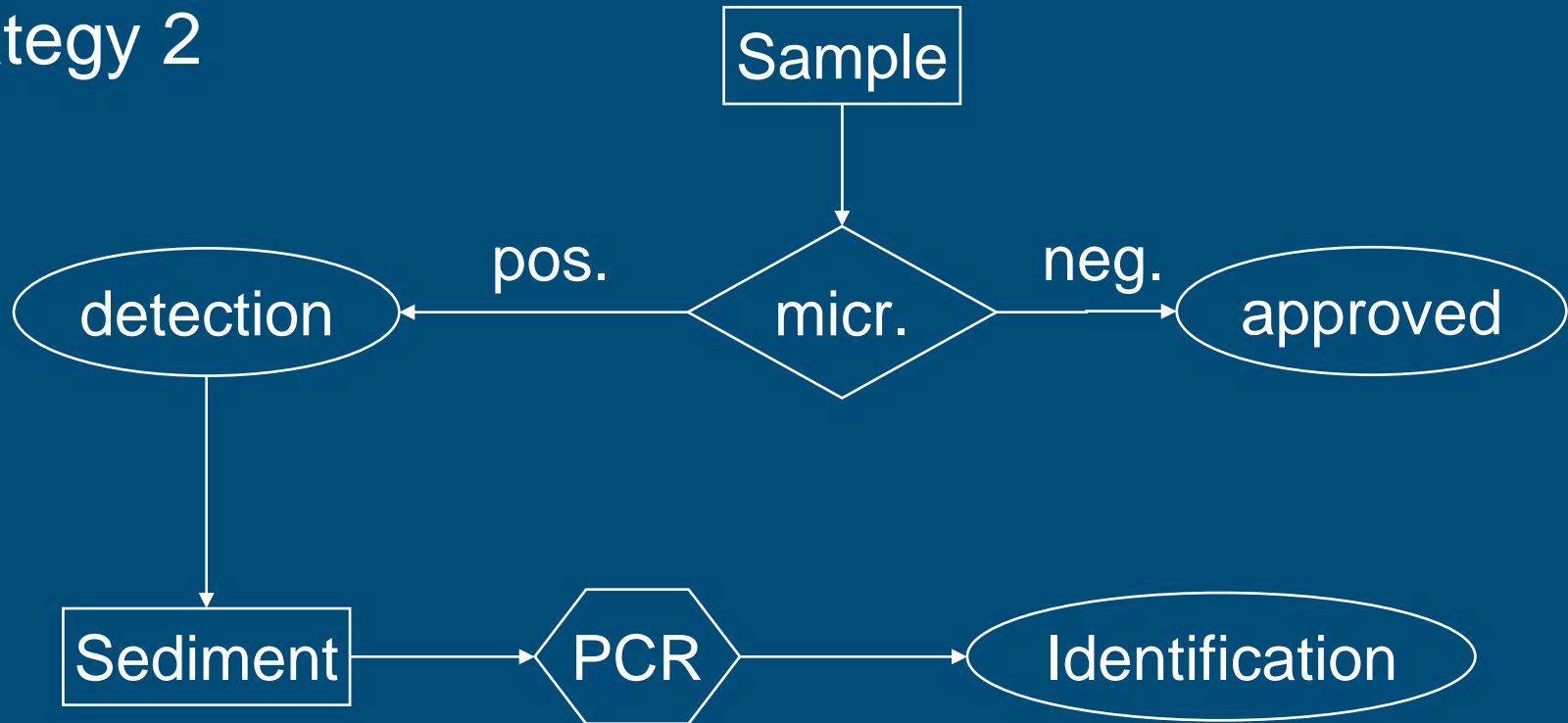
Detection of animal proteins

■ Strategy 1



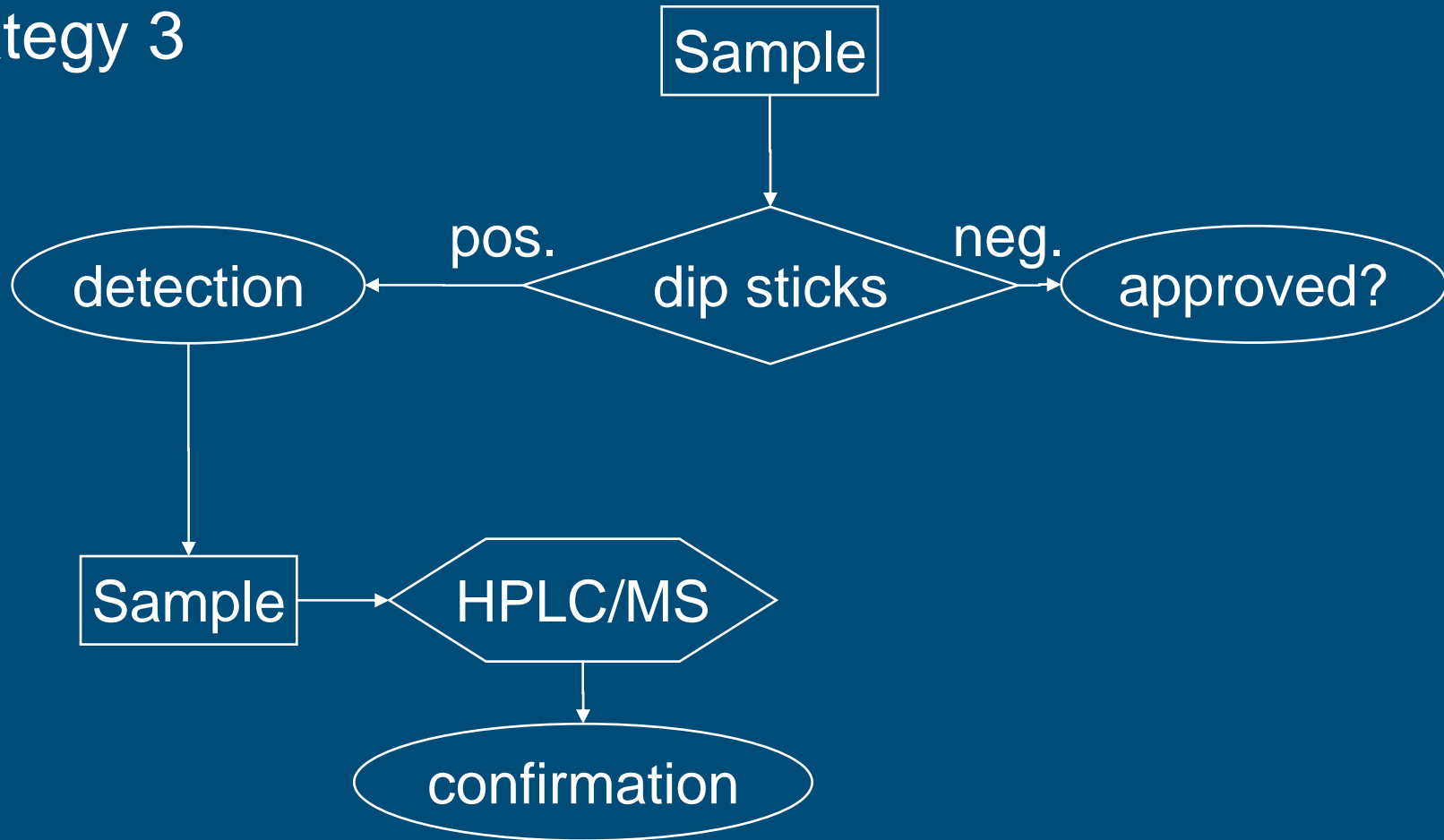
Detection of animal proteins

■ Strategy 2



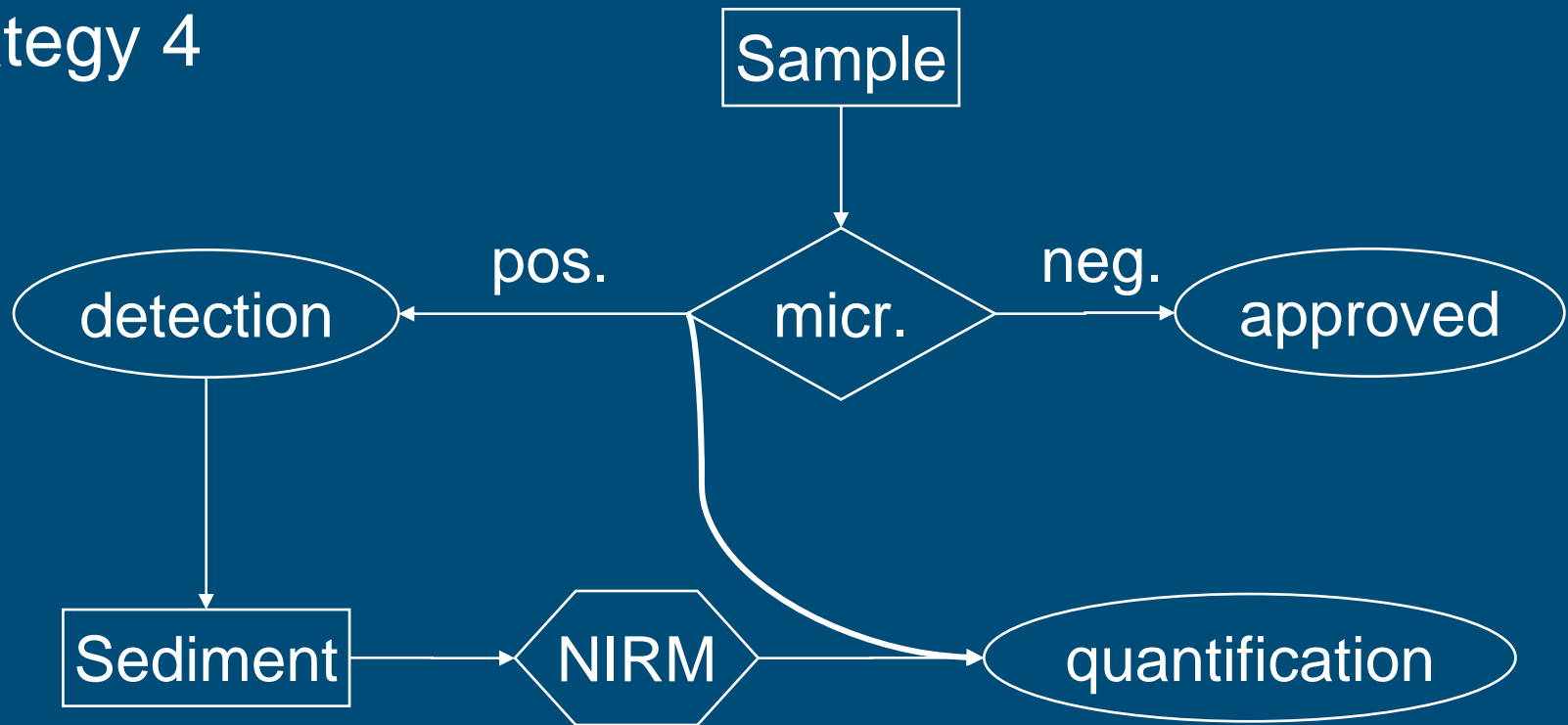
Detection of animal proteins

■ Strategy 3



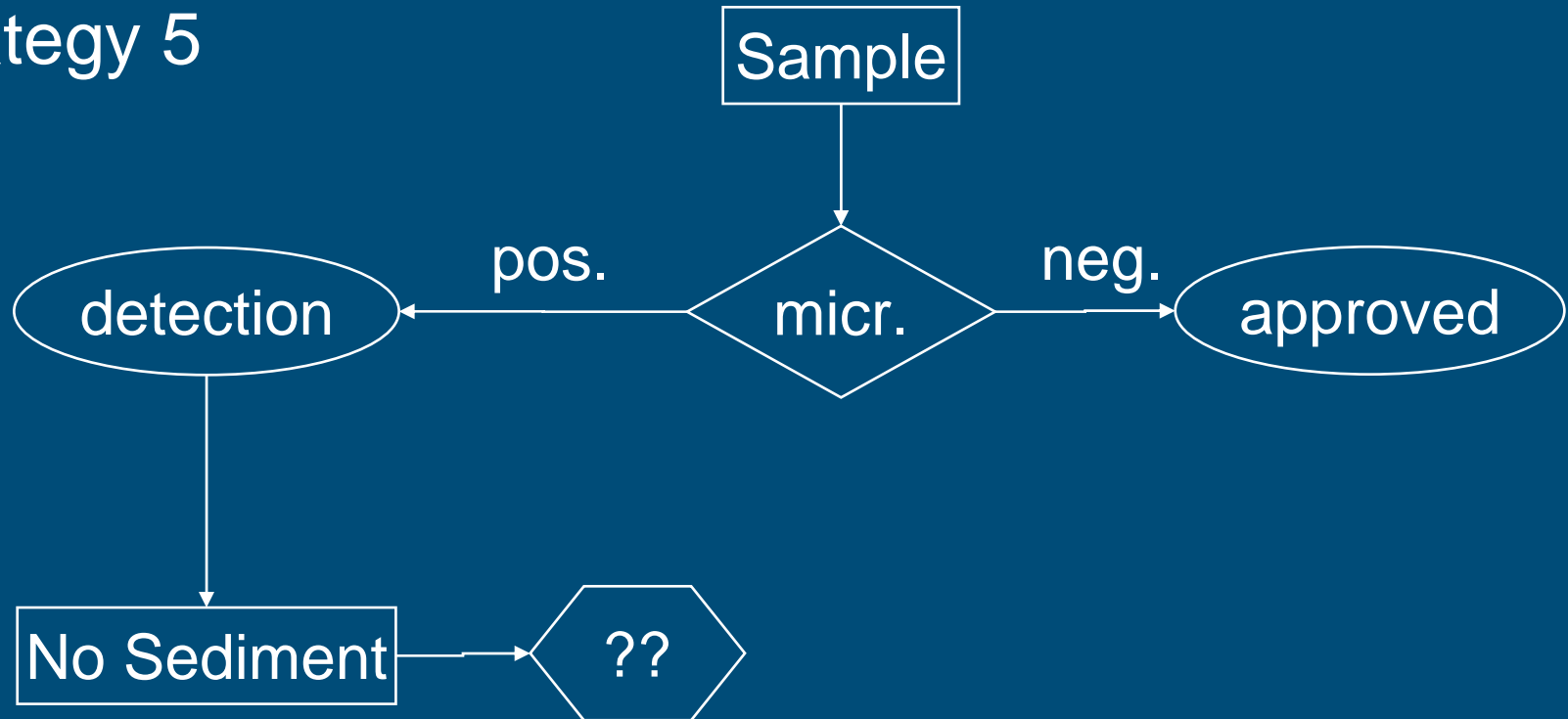
Detection of animal proteins

■ Strategy 4



Detection of animal proteins

■ Strategy 5



Detection of animal proteins

- Combination method

- ⇒ No sediment: no bones

- ⇒ Most prominent alternative tissue: muscle

- ⇒ Antibodies available or in development

Detection of animal proteins: microscopy

■ Procedure

- Concentration of (possibly present) muscle fibres
- Preparation of slides
- Blocking: ovalbumine and pre-treatment
- Conjugation with species-specific antibody
- Conjugation with second antibody-enzyme system
- Final embedding and examination
- *Several intermediate washing steps*

Detection of animal proteins

- Combination method

- ↳ Concentration of (possibly present) muscle fibres

- TCE :
Heptane 70 :
30



Detection of animal proteins

■ Combination method

↳ Production of slides

- Adhesive: Norland 81
- Good distribution of material on slide
- Hardening of Norland with UV light
- Handling (rinsing etc.) possible
- Storability: at least three weeks

Detection of animal proteins

■ Combination method

↪ Blocking: ovalbumine and pre-treatment

- 0.05 – 1.0 % Triton in phosphate buffer

Detection of animal proteins

■ Combination method

↪ Conjugation with species-specific antibody

- AbD Serotec
- Chinese antibody: 1G11
- RIKILT antibody: 86-2F1G2F10

Detection of animal proteins

■ Combination method

↪ Conjugation with second antibody-enzyme system

- Several fluorochromes
- Vector Blue staining Kit (Vector Lab SK-5300)
- Amino Ethyl Carbazol (AEC+; DAKO K3461)

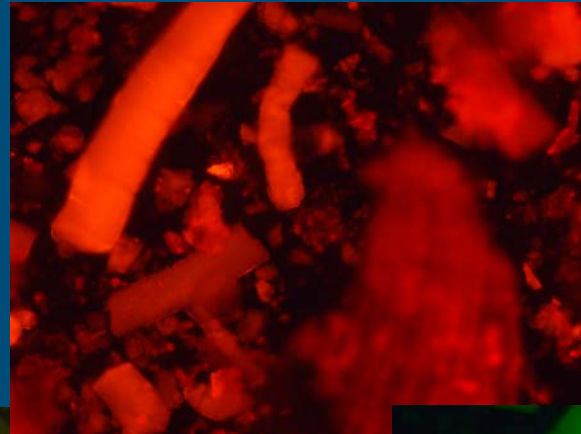
Detection of animal proteins

■ Results 1: autofluorescence

bright
field



WIG



WIB



WIBA



Detection of animal proteins

- Results 2: AbD Serotec, Vector Blue, porcine



Detection of animal proteins

- Results 3: AbD Serotec, AEC+, porcine



Detection of animal proteins

- Results 4: RIKILT Ab, Vector Blue



bovine



bovine



porcine

Detection of animal proteins

- Results 5: Chinese Ab, Vector Blue



bovine



bovine



porcine

Detection of animal proteins: **microscopy**

■ Strengths and weaknesses

detection level



heat resistance



interfering ingredients



required expertise level



reliable identification



time consumption



financial investment



Detection of animal proteins: immunoassays (ELISA)

■ Strengths and weaknesses

detection level



heat resistance



interfering ingredients



required expertise level



reliable identification



time consumption



financial investment



Detection of animal proteins: combination method

■ Strengths and weaknesses

detection level



heat resistance



interfering ingredients



required expertise level



reliable identification



time consumption



financial investment



Detection of animal proteins: ARIES



■ Decision Support System ARIES

- Support of official control

- protocol, identification keys, documentation

- Training

- glossary, gallery, observation log

- Copies available; special price

Thank you

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