AXOScience

STK Spray validation guide, from STK Sperm Tracker™ range according to the COFRAC recommendations

Preamble: This technology has already been validated by COFRAC in several French laboratories.

Methodology:

- **Presentation of the technique, apparatus and procedure, field of application and purpose of the verification/method validation**
- **Determination of performance criteria (parameters) to be checked:**
  - **Sensitivity, repeatability, intermediate fidelity**
  - **Specificity and interactions with other fluids**
  - **Performance on different supports**
- **Determination of acceptable specifications or limits (objectives to be achieved) of these criteria**
- **Bibliographic verification**
- **Experimental design and experimental implementation in the laboratory**
- **Compilation and statistical processing of the data obtained**
- **Conclusion and decision on the operational validation of the technique, with regard to the specifications (acceptable limits) initially set**

Design of experiment and experimental implementation in the laboratory

1. **Sensitivity, repeatability and intermediate fidelity test: Sample preparation: 50 µL deposits**
   a. Pure semen
   b. Diluted semen (1/2, 1/5, 1/10, 1/100, 1/1000)
   c. Negative control: water

➔ Deposit in **triplicate** of **pure sperm** and **different dilutions of sperm** on the same support such as glass benchtop or KIMTECH type paper, then drying for at least 4 hours.

1st deposit

NC	Pure semen	Semen 1/2	Semen 1/5	Semen 1/10	Semen 1/100	Semen 1/1000

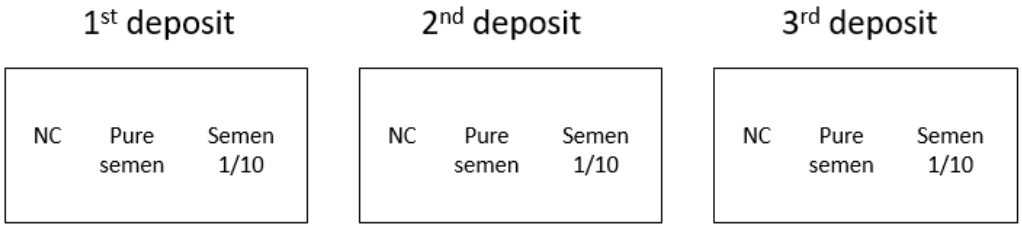
2nd deposit

NC	Pure semen	Semen 1/2	Semen 1/5	Semen 1/10	Semen 1/100	Semen 1/1000

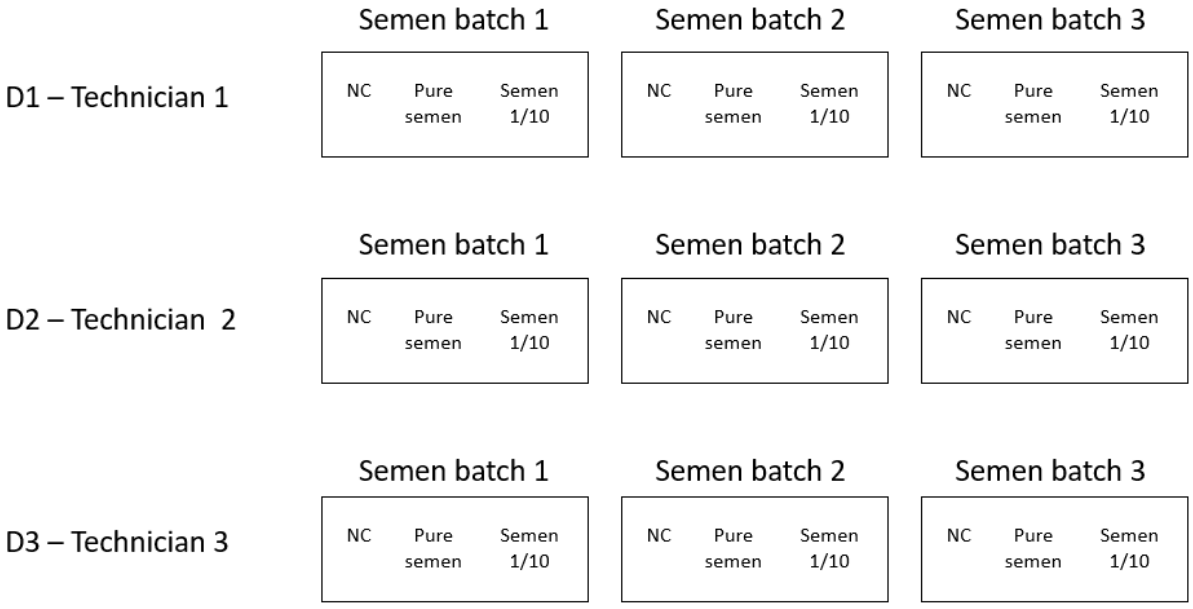
3rd deposit

NC	Pure semen	Semen 1/2	Semen 1/5	Semen 1/10	Semen 1/100	Semen 1/1000

➔ Deposit in **triplicate** of **pure sperm** and **diluted to 1/10** on the same support such as glass benchtop or KIMTECH type paper, then drying for at least 4 hours.



➔ Deposit in **triplicate** of **pure sperm** and **diluted to 1/10** from 3 different batches of sperm on the same support such as the glass bench or kimtech type paper, then drying for at least 4 hours. The experiment will last for 3 days knowing that each day, a different operator will spray the solution on the three deposits (negative control, pure semen and semen diluted to 1/10) for each batch (9 deposits in total per day for one operator).



2. **Specificity and interactions test: Sample preparation: 50 µL deposits**
    a. Pure semen
    b. Pure saliva
    c. Pure urine
    d. Pure blood
    e. Pure faeces
    f. Pure semen and pure blood
    g. Pure sperm and pure saliva
    h. Pure semen and pure urine
    i. Pure semen and pure faeces
    j. Negative control

➔ Deposit in **triplicate** of the **different fluids** on the same support such as the glass bench or paper type KIMTECH, then drying for at least 4 hours

1st deposit

NC	Pure semen	Pure blood	Pure saliva	Pure urine	Pure faeces	Pure semen + pure blood	Pure semen + pure saliva	Pure semen + pure urine	Pure semen + pure faeces

2nd deposit

NC	Pure semen	Pure blood	Pure saliva	Pure urine	Pure faeces	Pure semen + pure blood	Pure semen + pure saliva	Pure semen + pure urine	Pure semen + pure faeces

3rd deposit

NC	Pure semen	Pure blood	Pure saliva	Pure urine	Pure faeces	Pure semen + pure blood	Pure semen + pure saliva	Pure semen + pure urine	Pure semen + pure faeces

3. **Performance test: Preparation of the support to be tested**
    a. Smooth (sink, toilet)
    b. Rough (concrete, wall)
    c. Car interior
    d. Waterproof (glass bench)
    e. Natural (wood)
    f. Plastic (hairbrush, pen etc.)

➔ **Triplicata** deposit of **pure semen** and the **different dilutions** of semen (1/10 and 1/100) on each of these supports, then drying for at least 4 hours.

	1st deposit	2nd deposit	3rd deposit
Smooth/hard material = bathroom sink	NC / Pure semen / Semen 1/10 / Semen 1/100	NC / Pure semen / Semen 1/10 / Semen 1/100	NC / Pure semen / Semen 1/10 / Semen 1/100
Rough material = concrete, roughcast wall	NC / Pure semen / Semen 1/10 / Semen 1/100	NC / Pure semen / Semen 1/10 / Semen 1/100	NC / Pure semen / Semen 1/10 / Semen 1/100
Car interior	NC / Pure semen / Semen 1/10 / Semen 1/100	NC / Pure semen / Semen 1/10 / Semen 1/100	NC / Pure semen / Semen 1/10 / Semen 1/100
Waterproof material = glass bench	NC / Pure semen / Semen 1/10 / Semen 1/100	NC / Pure semen / Semen 1/10 / Semen 1/100	NC / Pure semen / Semen 1/10 / Semen 1/100
Natural material = wood, leaf	NC / Pure semen / Semen 1/10 / Semen 1/100	NC / Pure semen / Semen 1/10 / Semen 1/100	NC / Pure semen / Semen 1/10 / Semen 1/100
Plastic material = hairbrush	NC / Pure semen / Semen 1/10 / Semen 1/100	NC / Pure semen / Semen 1/10 / Semen 1/100	NC / Pure semen / Semen 1/10 / Semen 1/100

4. **Preparation of the STK Spray solution**
   a. Open a STK Spray pouch and pour it into a sprayer
      - The sprayer must allow to vaporize in fine misting
   b. Mix the powder with 100 mL of demineralized water
   c. Homogenize the solution until the powder dissolves completely (about 45 seconds)
   d. Prime the sprayer by pressing the trigger 2-3 times towards a trash can or sink

5. **Use of different UV lamps if possible to compare the difference in lamp performance with respect to signal detection.**
   Contact AXO Science to find out if your lamp model is suitable.
   365 nm UV lamp used and recommended by AXO Science: VILBER VL-6.L.
   a. 365 nm UV lamp → Characteristics to be respected:
      - Portable or plugged into the power supply(at your convenience)
      - Specificity with sperm once STK Spray sprayed
      - Signal to background noise ratio (very little interference): specific and intensity
      - Signal expression (the stain) + easy to distinguish compared to areas devoid of sperm
      - High power of the UV lamp
      - If neon tube lamp: length of the neon (neon tube quite long with a larger surface to be observed)
   b. Protect your eyes well with transparent UV-resistant glasses

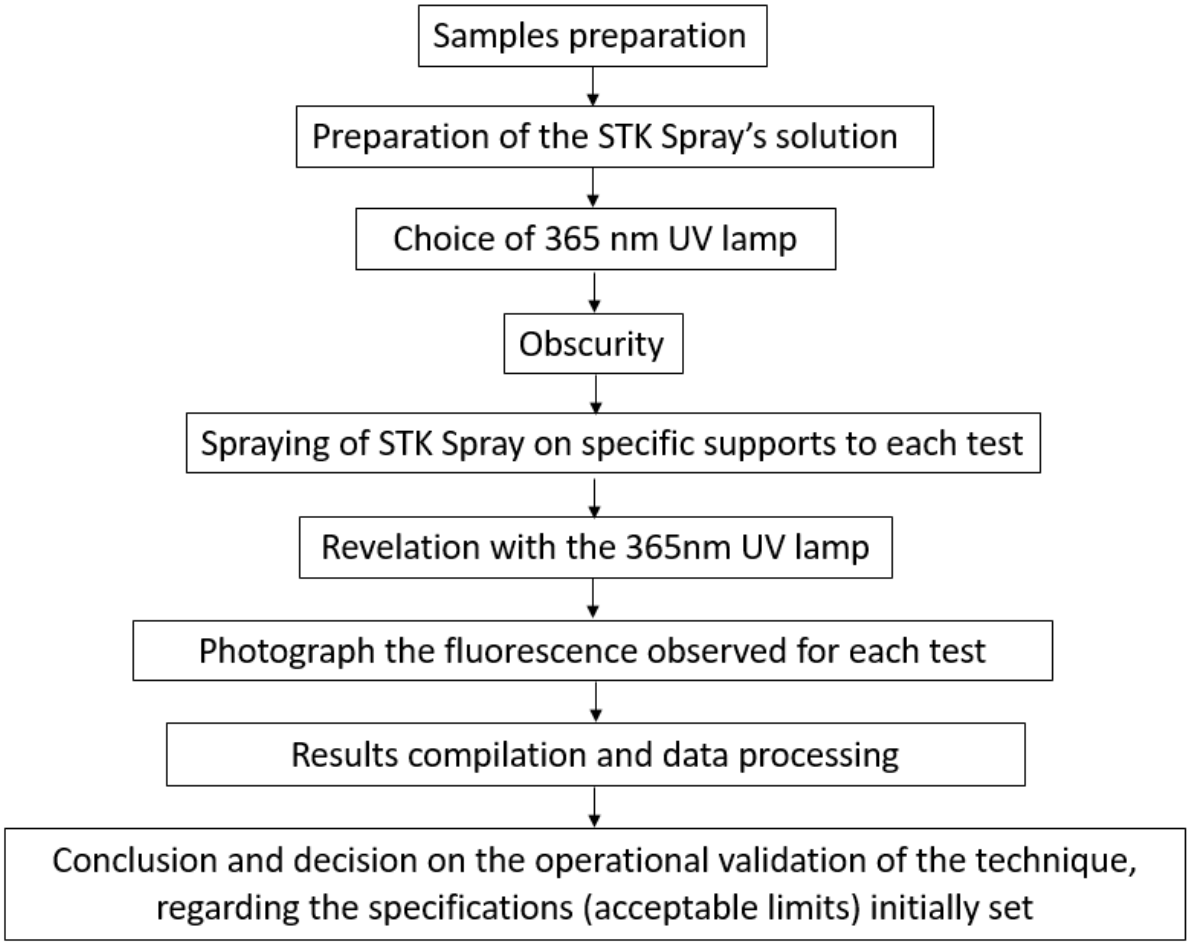
6. **Tests realizations**
   a. Being in the dark
   b. Spray the different supports using the sprayer containing the solution by keeping it vertical
   c. After waiting between 45 seconds to 1 minute, illuminate the samples with the 365nm UV lamp (or UV lamps if lamp comparison study):
      - for sensitivity test
      - for specifity test
      - for performance test
   d. Take pictures of the results obtained with a camera whose settings are fixed for each element to be photographed.

Example for a CANON EOS 2000D camera that is attached to a steel box allowing you to be as much as possible in the dark:

- ***Focal: F5.6***
- ***ISO: 3200***
- ***Shutter speed: 1/20th of a second***

7. **Results compilation and data processing**

8. **Conclusion and decision on the operational validation of the technique, against the specifications (acceptable limits) initially set.**


Samples preparation
Preparation of the STK Spray's solution
Choice of 365 nm UV lamp
Obscurity
Spraying of STK Spray on specific supports to each test
Revelation with the 365nm UV lamp
Photograph the fluorescence observed for each test
Results compilation and data processing
Conclusion and decision on the operational validation of the technique, regarding the specifications (acceptable limits) initially set