INNO4VAC

Innovations to accelerate vaccine development and manufacture

Annual Meeting 2023

Standard Operating Procedure (SOP) template

WP12
Sciensano
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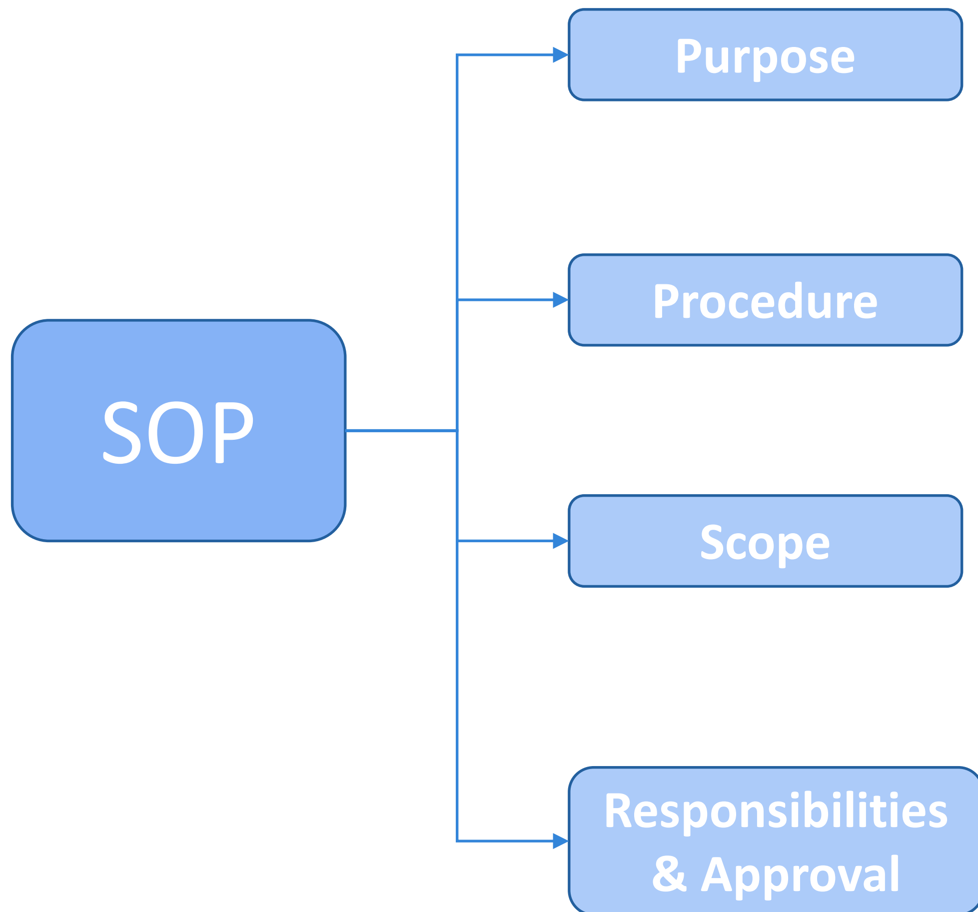
What is an SOP ?



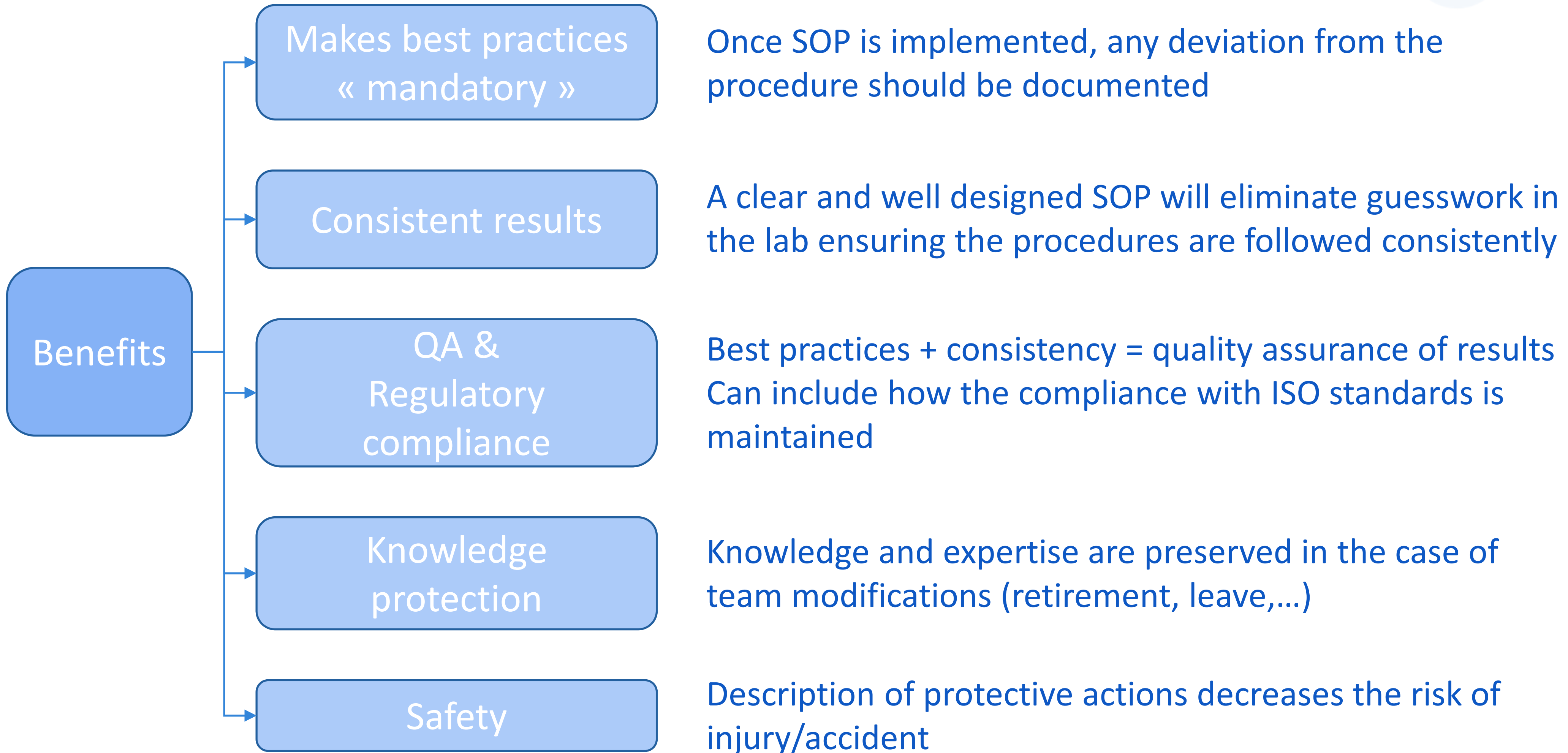
Define the goals of the SOP and the general principle of the experiment

Provide clear step by step instructions for routine operations

Explain the range of activities to which the SOP apply and the limits/exceptions to which it does not

Specificy who participated in the writing of the document, who approved the final version/change (revision history), and who is in charge of what in the procedure

Why do you need an SOP?



Helpful for:
standardization
validation
transferability

Example of unclear procedure

Isolation of human SSCs from OA patients

Testicular tissues from 60 OA patients for at least 7 experiments were washed three times in DMEM-containing antibiotics penicillin and streptomycin. Seminiferous tubules were isolated from human testis biopsies using 2 mg/ml collagenase IV (Sigma) and 1 μg/μl DNase I (Gibco). Human testicular cells were obtained using a second enzymatic digestion with 4 mg/ml collagenase IV, 2.5 mg/ml hyaluronidase (Sigma), 2 mg/ml trypsin (Sigma), and 1 μg/μl DNase I. For differential plating, cells were seeded into culture plates in DMEM/F-12 (Gibco) supplemented with 10% FBS (Hyclone) and incubated at 34 °C in 5% CO2 for 12 h. After incubation, Sertoli cells attached to the culture plates, whereas male germ cells were remained in suspension and collected by centrifugation at 1000 rpm for 5 min.

Size of tissue ? Should I cut it ?
Which DMEM ? Antibiotic concentration ?

How to isolate tubule? Cutting, pippeting,.. ?
References of reagents ?

Enzyme references ?
Medium used for enzyme preparation ?

Which culture plates ?
Media & FBS reference ? (dozen of DMEM/F-12 on Gibco website)

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Sun, M., *et al.* Efficient generation of functional haploid spermatids from human germline stem cells by three-dimensional-induced system. *Cell Death Differ (IF=13.3)*, 2018

Experimental protocol *vs.* SOP

	Experimental protocol	**SOP**
Scope	Individualized guidelines/routines created for personal use	Formalized procedure use by an organisation to ensure consistency
Purpose	Help for time and task management	Standardization and streamlining of processes
Formality	Informal and flexible Can be adapted according to preferences/needs	Follows a specific format, require approval from relevant person
Applicability	Allows for adjustments based on the individual's preferences or the specific requirements of an experiment	Rigid Must be followed exactly as written to ensure consistency

SOP: Table of contents

1. Definitions and abbreviations
2. Principle of the model/method
3. Security
4. Material
   a) Reagents
   b) Consumables
   c) Equipment
5. Biological material recovery and preparation

Describe the principle of your model
e.g. « This doc aimed to describe the protocol for generation of intestinal organoids from hiPSCs »

List the precautions to be taken
e.g. "wear gloves/apron/safety glasses, manipulation of the strain xx in L3"

List all the required reagents (in-house, commercial, references)
e.g. « Homemade culture media composed of DMEM + … », « Commercial trypsin/EDTA from… », « International serum reference from NIBSC »…

List the origin of all biological material
e.g. donor origin, tissue origin, transport conditions

Describe the protocol for preparation of primary cell/tissue source(s) prior to organoid, ALI or on chip culture. If a cell isolation, amplification and/or differentiation step is needed before the 3D model generation

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SOP: Table of contents

6. Biological material storage
7. Experimental part
   a) 3D model generation and characterization
   b) 3D model infection
   c) Addition of the immunological components
   d) Cryopreservation
   e) Readout

Explain the procedure for the storage of the biological material
e.g. cryopreservation protocol of primary cells, organoids,…

Describe how the model is built and how it is characterized to confirm that it is ready to be used for subsequent testing
e.g. cell identity, diameter, area of measurement, proliferation rate,…

Describe how the pathogen (virus, bacteria) is added to the culture
e.g. concentration, timepoints, microinjection…
Bacteria or virus seed banking should be described as well

Describe how the immune cells are added to the culture
e.g. cell density, timepoints, microinjection…

Describe the methodology for obtaining the different readouts
e.g. cytokine and LDH assays, virus titration, gene or protein expression,…

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SOP: Table of contents

8. Result validation
9. Calculations
10. References

Explain the acceptance criteria to whom the results should answer and methodology to set them
e.g. clinical relevance, statistical analysis…
Criteria can be established for the cell characterization
e.g. genes or cell markers expression to confirm the state of differentiation
Acceptance criteria can depend on the output analysis
e.g. cytokine secretion pre/post infection, detection of pathogen amplification, inhibition of replication following antisera or neutralizing antibody
Blanks, positive and negative controls should be established

Describe the calculations leading to results and if applicable the software used

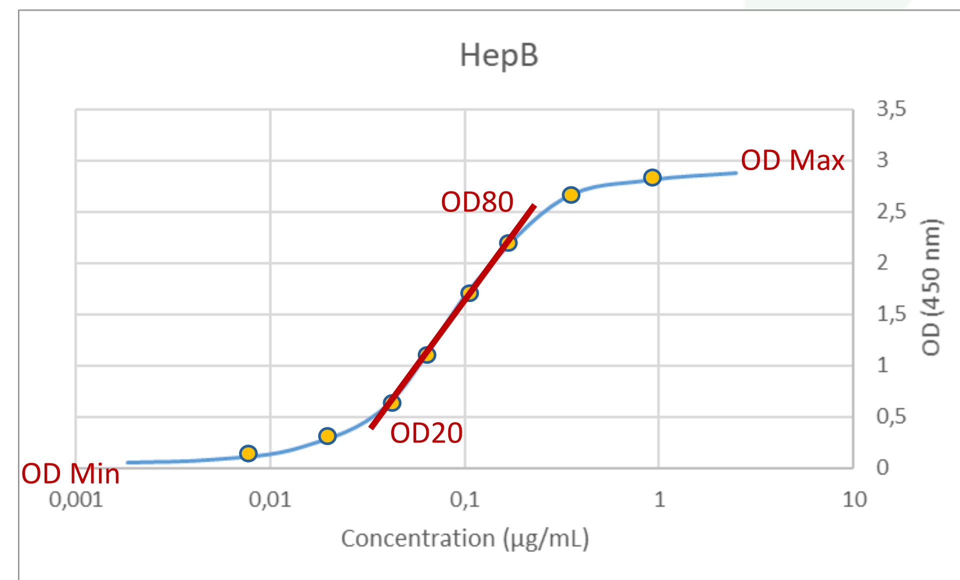
Explain the norms and references on which the methods rely
e.g. standardized methods/protocols, scientific articles, in-house SOP, international guidelines, Pharmacopeia…

Example of criteria for an ELISA

HepB antigen titration for vaccine batch release

Results evaluation/approval:

1. Standard curve
    a) $R^2 \geq 0.95$
    b) OD80 – OD20 ≥ 1
    c) Intradilution CV ≤ 20%
    d) At least 3 dilution points between OD80 and OD20
    e) -3SD ≤ Slope ≤ +3SD (of established specifications)
2. Internal control
    a) Titer determined with at least 3 valid values (comprised between OD80-OD20)
    b) Interdilution CV ≤ 20%
    c) -3SD ≤ titer ≤ +3SD (of established specifications)
3. Samples
    a) Titer determined with at least 3 valid values (comprised between OD80-OD20)
    b) Interdilution CV ≤ 20%



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Example of criteria for a PCR

Detection and quantification of parvovirus B19 in human plasma samples

Results evaluation/approval:

1. Standard curve
    a) $R^2 > 0.98$
    b) -3.74< slope <-3.00
    c) 85%< Efficiency < 115
    d) Δ Ct between replicates < 0.6
2. Controls
    a) Ct Negative extraction control > intercept of the equation of the standard curve
    b) Ct NTC > intercept of the equation of the standard curve
    c) Ct < 34 for positive control
    d) Presence of an internal control signal (JOE fluorochrome) in all wells
3. Samples
    a) Δ Ct between replicates < 0.6

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Example of material & reagent lists

6.5 *Lab equipment*

- Vortex
- Micropipettes P5, P10, P20, P50, P200 et P1000
- Sterile tips
- Freezer -20°C ± 5°C
- Fridge 2-8°C
- Disposable gloves
- Lab coat
- RNase away (VWR, Cat n° 732-2352)
- Spray umomium
- 10% bleach
- Agilent 4200 Tapestation
- Centrifuge
- Minifuge
- Plate spinner
- Autoclaved eppendorf tubes 1.5-2ml
- Tubes PCR 0.2 ml (Life technologies, Cat. n° 4316567)
- PCR strips (Life technologies, Cat. n° 4323032)
- Thermocycleur en temps réel QuantStudio 6 Flex system
- Plaques : MicroAmp optical 96 well (10 ; Thermofisher ; N° Catalogue N8010560)
- MicroAmp optical adhesive film (100 ; Thermofisher, N° Catalogue 4360954)
- TapeStation loading tips, 10 Pk (Agilent Technologies, Cat. n° 5067-5599)
- Tapestation Optical tube strips (8x Strip) (Agilent Technologies, Cat. n° 401428)
- Tapestation Optical tube strip caps (8x strip) (Agilent Technologies, Cat. n° 401425)
- Tapestation 96-well Sample Plates (Agilent Technologies, Cat. n° 5042-8502)
- Tapestation 96-well plate foil seal (Agilent Technologies, Cat. n° 5067-5154)
- PCR cooler
- ~~Water bath~~
- Thermomixer
- Gel tray & gel caster
- Gel electrophoresis system (Bio-Rad)
- Microwave
- Balance
- Glass Erlenmeyer
- Gel imaging system

→ List all equipments needed for performing the procedure

→ Allows to anybody to check what he needs to **reproduce** the procedure

7.2. Reagents
7.3. Solutions to prepare
7.3.1. 1% poloxamer 188
7.3.2. 0.001% Poloxamer 188
7.3.3. 1mg/ml E. coli tRNA Stock
7.3.4. 0,1mg/ml Yeast tRNA Stock
7.3.5. assay diluent
7.3.6. Primer reconstitution
7.3.7. Preparation of optimized primer mix (OPM)
7.3.8. Probe reconstitution
7.3.9. Preparation of optimized primer-probe mix (OPPM)

→ Listing of all reagents and solutions

When to write your SOP ?

- Once your procedure is **technically validated**, a final version of the SOP can be considered

- As a first step → a draft SOP can be a good start if your model is not likely to undergo major changes
  - Gather all steps of the procedure
  - List the required reagents, consumables, equipments
  - Facilitate the transfer of the method with a potential partner/collaborator, the training of new team member

Take home message

- Start writing your (draft) SOP as soon as you can
- Minimize all sources of variability not linked to your model (human error, variations)
- Serve as a guideline for training and method transfer

<u>SOP template available on Inno4Vac sharepoint:</u>

“Documents > 02. Governance > 5. STs > ST3 > ELECTRONIC PORTAL - ST3 model protocols and validation”