## SOP: Standard Operating Procedures for work with:

**Diphtheria Toxin and Diphtheria Toxin-treated Animals**

University of Washington

<table>
<thead>
<tr>
<th>Standard Operating Procedures for Chemicals or Processes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>#1 Preparation</strong></td>
</tr>
<tr>
<td>If possible do not work with powder form of diphtheria toxin (DT). If necessary purchase pre-weighed or pre-diluted DT in the least quantity possible to perform work. <em>[Vials of DT will be purchased in pre-weighed powder form and then reconstituted in a fume hood/glove box/biological safety cabinet (BSC)]. Weighing the toxin is not necessary as reconstitution will occur in the purchased vial and then aliquoted into vials with caps]</em>.</td>
</tr>
<tr>
<td>*<em>Vaccine is available for diphtheria, and must be offered every ten years. If persons working with DT are not current with their immunizations, contact the UW Employee Health Center (EHC) at 206-685-1026.</em></td>
</tr>
</tbody>
</table>

| **#2 Chemicals/Hazards** |
| DT can be extremely toxic at very low levels. All contact should be avoided. Symptoms of exposure include skin irritation, respiratory irritation, fever and headache. Do not breathe dust, fume or vapors of DT powder or solutions. DT may cause death if ingested. |

| **#3 Personal Protective Equipment (PPE)** |
| Laboratory coat or gown with long sleeves |
| Eye/face protection: safety glasses with side shields or chemical safety goggles, face protection such as a face shield if splash/spatter possible |
| Double gloving with thin nitrile gloves. Change gloves and dispose immediately if contaminated, torn or punctured. |

| **#4 Environmental/Ventilation Controls** |
| Containers with DT must be handled in a *fume hood or BSC*. Signs will be posted on the room door and *fume hood or BSC* when toxins are in use stating: “Toxins in Use. Authorized Personnel Only” |

| **#5 Special Handling Procedures & Storage Requirements** |
| **HANDLING** |
| **Prep** |
| • All preparation of DT will be performed over plastic backed absorbent pads. |
| • Reconstitution, dilution and administration of the toxin will be performed only in a *fume hood or BSC* while wearing PPE. |
| **Use** |
| • Only needle locking (Luer-Lock type) syringes or disposable syringe needle units are used for injection or aspiration of infectious or biohazardous material. |
| • A sharps container will be in the immediate vicinity for safe sharps disposal. |
| • Hands will be washed upon completion of tasks. |
| **STORAGE** |
| • Unused DT will be kept in its original container or aliquoted into labeled vials that are tightly closed and stored at -20°C in CD 186L. The original DT container or vials containing aliquots will be stored inside a labeled, leak-spill-proof secondary container. |
| • Store DT in a secure location. |
| **TRANSPORT** |
| • Transport DT in secondary, sealed, labeled non-breakable containers. |
If you are not trained or comfortable cleaning up a spill, call 206-543-0467 for the EH&S spill hotline for assistance. If it is an emergency (risk of exposure to others such as an on-going DT release) call 911.

**Liquid spills**: To be cleaned by properly protected and trained personnel. Personnel cleaning up a liquid spill will wear a lab coat/gown, goggles, and two pairs of nitrile gloves. Cover spill with absorbent paper towels and apply 1% sodium hypochlorite (NaOCl) (or 10% bleach), starting at the perimeter and working towards the center, allowing 30-min. contact time to deactivate DT before clean up. Clean the spill area with 1% NaOCl (or 10% bleach) allowing 30 min. contact time, then soap and water. The decontaminated spill waste will be double bagged and disposed of in regular trash.

**Powder spills inside of fume hood or BSC**: To be cleaned by properly protected and trained personnel. Personnel cleaning up a powder spill will wear a lab coat/gown, goggles, and two pairs of nitrile gloves. Gently cover powder spill with dampened absorbent paper towels to avoid raising dust. Apply 1% NaOCl (or 10% bleach), starting at the perimeter and working towards the center, allowing 30-min. contact time to deactivate DT before clean up. Clean the spill area with 1% NaOCl (or 10% bleach) allowing 30 min. contact time, then soap and water. The decontaminated spill waste will be double bagged and disposed of in regular trash.

Wash hands thoroughly after completing any spill clean up.

**Powder spills outside of a fume hood or BSC**: Remove all personnel from the room and restrict access. A spill cleanup contractor will need to manage the spill since adequate respiratory protection is not available.

As soon as possible report the spill by notifying EH&S (EH&S business hours 206-543-0467, outside business hours 206-685-8973 (UWPD); tell them that a spill has occurred, and you need EH&S to obtain a spill cleanup contractor. Be prepared to provide the following information:

- Name and phone number of knowledgeable person that can be contacted: Jennifer Stone: 206-616-4108
- Name of chemical, concentration and amount spilled: Diphtheria Toxin
- Number of injured, if any: ____________
- Location of spill: ___________________

This information can also be used in reporting to the Emergency Department after potential exposure.

Any spill incident requires the supervisor to complete and submit the online accident reporting system (OARS) form within 24 hours of the incident to EH&S.

**First Aid Procedures**:

- **For oral (mouth) exposure** or if DT has been swallowed and if the person is conscious, wash out mouth with water while another worker calls 911 on campus phone if available (landline phone tracks the person’s location for emergency medical responders.)
- **For inhalation exposure**, move person to fresh air. If breathing becomes difficult call 911.
- **For contact exposure** to the eye, flush eye with copious amounts of water for at least 15 minutes and call 911.
- **For dermal exposure**, rinse area with copious amounts of water for at least 15 minutes, remove any contaminated clothing. **Call 911, or go directly to Emergency Department (ED).**

- **Needlesticks are a medical emergency** and all work should be halted, with another person securing things while the injured person washes and obtains treatment. **Call 911, or go directly to ED.**

**After First Aid Treatment:**
Where to Call if non-life-threatening emergency

- If a non-life-threatening exposure incident occurs, do the first aid as described above, then

  - Contact the UW Employee Health Clinic (206-685-1026). The nurse will advise whether to be seen at EHC or the ED.

  - If it has been 5 years or greater since the person received diphtheria vaccine, a booster may be recommended.

BRING TO THE ED: the DT MSDS and this entire SOP, including the Emergency Dept. information at the end of this document.

Any exposure incident requires the supervisor to complete and submit the online accident reporting system (OARS) form within 24 hours of the incident to EH&S.

#7 Waste Disposal and Cleaning

Any waste DT will be decontaminated or autoclaved before disposal whenever possible.

Work space surfaces must be wiped down with 1% NaOCl, 10% bleach or 1N NaOH daily, during the length of the experiment. To prevent corrosion of metal surfaces rinse with water after using chlorine-based chemicals. Absorbent pads will be replaced daily. The used and contaminated absorbent pads, PPE, etc. will be placed in a biohazard bag and autoclaved. Note that some disinfecting agents may not deactivate DT.

If in-lab decontamination/autoclaving is not possible for some DT waste, it should be managed as chemical waste. For chemical waste pick up: Complete a Chemical Collection Request Form and scan, fax or mail it to: chmwaste@uw.edu, FAX to 206-685-2915, or mail to Box 354110. A chemical collection request form can be found at www.ehs.washington.edu/chemical/hazardous-chemical-waste-disposal.

#8 Special Precautions for Use of DT in Animals (if applicable)

Be extremely cautious using needles with DT. Follow the written procedures for safe use of sharps, and practice doing a “dry run” with less hazardous materials as needed. A sharps container must be in the immediate vicinity for safe sharps disposal.

DO NOT RECAP needles. Never leave exposed needle tip in work area. Use a syringe holder to secure syringe. Contact EH&S for consultation at 206-221-7770. Perform procedures in a [fume hood or BSC] while wearing double nitrile gloves, lab coat/gown, safety glasses/goggles and face protection such as a face shield if splash/spatter possible.

Animals will be anesthetized or placed into a restraining apparatus before procedures using DT are performed. Once the animal has been properly fitted into the restraining apparatus, the syringe will be loaded just prior to injection.

Hands must be washed with soap and water after completion of the injection, and then put on new pair of nitrile gloves. If continuing to inject more animals, remove gloves and wash hands afterwards.

After procedures are complete, the restraining apparatus and surrounding work station will be decontaminated using 1% NaOCl, 10% bleach or 1N NaOH. All reusable lab equipment will be autoclaved.

No special disposal requirements are needed for animals that have received DT.

<table>
<thead>
<tr>
<th>Particular hazardous substance involved?</th>
<th>X YES: Blocks #9 to #11 are Mandatory</th>
<th>NO: Blocks #9 to #11 are Optional</th>
</tr>
</thead>
</table>

#9 Approval Required

All staff working with DT must be trained on this SOP prior to starting work. They must also be trained on the DT MSDS and it must be readily available in the laboratory. All training must be documented and maintained by the PI.
#10 Decontamination
All surfaces will be decontaminated with 1\% NaOCl, 10\% bleach or 1N NaOH after removing the plastic backed pads. **Note that bleach solutions should be prepared fresh daily.** All reusable lab equipment will be autoclaved. Note that some disinfecting agents may not deactivate DT.

#11 Designated Area
All work with DT must be done in a designated laboratory, work space and [fume hood or BSC]. Signage must be placed in work spaces and on [fume hood or BSC] when DT is used. This work will be conducted in Room(s): CD 186K or 186A, CD 055.

| Name:   | Dr.s Edwin W Rubel and Jennifer Stone | Title: Professors | Signature: [Signature] | Date: 02/17/2022 |

**EMERGENCY DEPARTMENT RESPONSIBILITIES (POST EXPOSURE TO DIPHTHERIA TOXIN)**

a. There have been reports of rapid onset of local pain after percutaneous exposure to diphtheria toxin and such an occurrence would indicate a significant exposure. Onset of symptoms following significant diphtheria toxin exposure would typically have onset delayed by days to weeks and are due to the inhibition of protein synthesis. The Emergency Department (ED) shall assess the severity of the exposure and take appropriate actions to include consultation with the Washington Poison Control System: PH 1-800-222-1222. Treatment with Diphtheria Antitoxin (DAT) may be considered in case of an especially severe or large exposure, even in the absence of symptoms.

b. DAT is currently available from the CDC. In the event of problems obtaining a response at any local or state level, the CDC Emergency Operations Center should be contacted at (770) 488-7100. Contraindications/Precautions in the use of DAT include a history of prior exposure to horse serum, prior history of serum sickness, or a history of asthma or hay fever, especially when near horses.

c. The ED should draw at least ten milliliters of serum and hold it for possible diphtheria toxin assay. This must be done before any treatment with diphtheria antitoxin.

d. Any patient seen in the ED and released should be given information about the potential for delayed onset of symptoms/toxicity. Any symptoms would be reason for emergent reevaluation. Any exposed individuals should also be referred to the UW Employee Health Clinic (206-685-1026) for follow-up care.

The **Emergency Department** will need to complete a Physicians Report of Injury Report L&I form. The ED physician needs to contact the Employee Health Clinic (206-685-1026) and report that an exposure has occurred.
1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product identifiers
Product name: Diphtheria Toxin, from Corynebacterium diphtheriae
Product Number: D0564
Brand: Sigma

1.2 Relevant identified uses of the substance or mixture and uses advised against
Identified uses: Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet
Company: Sigma-Aldrich
3050 Spruce Street
SAINT LOUIS MO 63103
USA
Telephone: +1 800-325-5832
Fax: +1 800-325-5052

1.4 Emergency telephone number
Emergency Phone #: (314) 776-6555

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture
GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)
Acute toxicity, Oral (Category 1), H300
For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 GHS Label elements, including precautionary statements

Pictogram

Signal word: Danger
Hazard statement(s)
H300: Fatal if swallowed.
Precautionary statement(s)
P264: Wash skin thoroughly after handling.
P270: Do not eat, drink or smoke when using this product.
P301 + P310: IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.
P321: Specific treatment (see supplemental first aid instructions on this label).
P330: Rinse mouth.
P405: Store locked up.
P501: Dispose of contents/container to an approved waste disposal plant.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none
3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

**Hazardous components**

<table>
<thead>
<tr>
<th>Component</th>
<th>Classification</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria Toxin from Corynebacterium diphtheriae</td>
<td>Acute Tox. 1; H300</td>
<td>90 - 100 %</td>
</tr>
</tbody>
</table>

For the full text of the H-Statements mentioned in this Section, see Section 16.

4. FIRST AID MEASURES

4.1 Description of first aid measures

**General advice**
Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

**If inhaled**
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

**In case of skin contact**
Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

**In case of eye contact**
Flush eyes with water as a precaution.

**If swallowed**
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

5. FIREFIGHTING MEASURES

5.1 Extinguishing media

**Suitable extinguishing media**
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
Nature of decomposition products not known.

5.3 Advice for firefighters
Wear self contained breathing apparatus for fire fighting if necessary.

5.4 Further information
No data available

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures
Wear respiratory protection. Avoid dust formation. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust. For personal protection see section 8.

6.2 Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.
7. HANDLING AND STORAGE

7.1 Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Keep container tightly closed in a dry and well-ventilated place.
Recommended storage temperature: 2 - 8 °C
Keep in a dry place.

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters
Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

Personal protective equipment

Eye/face protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove’s outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Body Protection
Complete suit protecting against chemicals. The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties
a) Appearance
   Form: powder, lyophilized
b) Odour
   no data available
c) Odour Threshold
   no data available
d) pH
   no data available
e) Melting point/freezing point
   no data available
f) Initial boiling point and boiling range
   no data available
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>g)</td>
<td>Flash point</td>
<td>no data available</td>
</tr>
<tr>
<td>h)</td>
<td>Evaporation rate</td>
<td>no data available</td>
</tr>
<tr>
<td>i)</td>
<td>Flammability (solid, gas)</td>
<td>no data available</td>
</tr>
<tr>
<td>j)</td>
<td>Upper/lower flammability or explosive limits</td>
<td>no data available</td>
</tr>
<tr>
<td>k)</td>
<td>Vapour pressure</td>
<td>no data available</td>
</tr>
<tr>
<td>l)</td>
<td>Vapour density</td>
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</tr>
<tr>
<td>m)</td>
<td>Relative density</td>
<td>no data available</td>
</tr>
<tr>
<td>n)</td>
<td>Water solubility</td>
<td>soluble</td>
</tr>
<tr>
<td>o)</td>
<td>Partition coefficient: n-octanol/water</td>
<td>no data available</td>
</tr>
<tr>
<td>p)</td>
<td>Auto-ignition temperature</td>
<td>no data available</td>
</tr>
<tr>
<td>q)</td>
<td>Decomposition temperature</td>
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</tr>
<tr>
<td>r)</td>
<td>Viscosity</td>
<td>no data available</td>
</tr>
<tr>
<td>s)</td>
<td>Explosive properties</td>
<td>no data available</td>
</tr>
<tr>
<td>t)</td>
<td>Oxidizing properties</td>
<td>no data available</td>
</tr>
</tbody>
</table>

9.2 Other safety information
no data available

10. STABILITY AND REACTIVITY

10.1 Reactivity
no data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
no data available

10.4 Conditions to avoid
no data available

10.5 Incompatible materials
Strong acids, Strong bases, Strong oxidizing agents

10.6 Hazardous decomposition products
Other decomposition products - no data available
In the event of fire: see section 5

11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity
no data available

Inhalation: no data available

Dermal: no data available

LD50 Intraperitoneal - mouse - < 0.001 mg/kg
LD50 Intravenous - mouse - 0.00001 mg/kg
LD50 Subcutaneous - mouse - < 0.001 mg/kg
Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitisation
no data available

Germ cell mutagenicity
no data available

Carcinogenicity
IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity
no data available

Reproductive toxicity - Hamster - Intraperitoneal
Effects on Fertility: Other measures of fertility
no data available

Specific target organ toxicity - single exposure
no data available

Specific target organ toxicity - repeated exposure
no data available

Aspiration hazard
no data available

Additional Information
RTECS: Not available

Fever, Headache

Stomach - Irregularities - Based on Human Evidence
Stomach - Irregularities - Based on Human Evidence

12. ECOLOGICAL INFORMATION

12.1 Toxicity
no data available

12.2 Persistence and degradability
no data available

12.3 Bioaccumulative potential
no data available

12.4 Mobility in soil
no data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Other adverse effects
no data available
13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
UN number: 3462  Class: 6.1  Packing group: I
Proper shipping name: Toxins, extracted from living sources, solid, n.o.s. (Diphtheria Toxin from Corynebacterium diphtheriae)
Reportable Quantity (RQ):
Marine pollutant: No
Poison Inhalation Hazard: No

IMDG
UN number: 3462  Class: 6.1  Packing group: I  EMS-No: F-A, S-A
Proper shipping name: TOXINS, EXTRACTED FROM LIVING SOURCES, SOLID, N.O.S. (Diphtheria Toxin from Corynebacterium diphtheriae)
Marine pollutant: No

IATA
UN number: 3462  Class: 6.1  Packing group: I
Proper shipping name: Toxins, extracted from living sources, solid, n.o.s. (Diphtheria Toxin from Corynebacterium diphtheriae)

15. REGULATORY INFORMATION

SARA 302 Components
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
Acute Health Hazard, Chronic Health Hazard

Massachusetts Right To Know Components
No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components
Diphtheria Toxin from Corynebacterium diphtheriae  CAS-No. Revision Date

New Jersey Right To Know Components
Diphtheria Toxin from Corynebacterium diphtheriae  CAS-No. Revision Date

California Prop. 65 Components
This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

16. OTHER INFORMATION

Full text of H-Statements referred to under sections 2 and 3.
Acute Tox.  Acute toxicity
H300  Fatal if swallowed.

HMIS Rating
Health hazard:  4
Chronic Health Hazard:  *
Flammability:  0
Physical Hazard  0

NFPA Rating
Health hazard:  4
Fire Hazard:  0
Reactivity Hazard:  0

Further information
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Preparation Information
Sigma-Aldrich Corporation
Product Safety – Americas Region
1-800-521-8956

Version: 5.5  Revision Date: 07/01/2014  Print Date: 08/27/2014
### Safety Training Log

**PI/Supervisor:** Jennifer Stone  
**Department:** Oto-HNS  

Documentation should include formal and informal safety discussions, including any internal meetings when the agenda includes any safety discussions. Log discussions on Personal Protective Equipment ventilation systems, glove box requirements, specific chemical hazards, MSDS access, chemical storage plans, etc. Attach training outline and other reference materials useful for training new personnel.

<table>
<thead>
<tr>
<th>Date</th>
<th>Trainer</th>
<th>Trainees</th>
<th>Description of Safety Training</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>08/29/2018</td>
<td>Robin Gibson</td>
<td>Stone Lab Members</td>
<td>Location and contents of Lab Safety Manual; SOP for Diphtheria Toxin; PPE requirements</td>
<td></td>
</tr>
<tr>
<td>8/29/18</td>
<td>Sophie Seo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/29/18</td>
<td>Jialin Sheng</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd Apr 18</td>
<td>E. Jhoocean</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>29/01/18</td>
<td>Sharada K.C.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Re** label binder  
- **Label** SOPS