

Staining Waste

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What is Microbiological Staining?

Microbiological staining:

- A technique used to better visualize cells, including bacteria and cellular components.
 - Gram stain
 - Acid fast stain
 - Simple stain

Common facilities that conduct staining:

- Hospitals and medical clinics
- Veterinary clinics and hospitals
- Diagnostic and histopathology laboratories
- Research laboratories such as those found at universities and the biotech industry

Common chemicals used in the staining process:

- Crystal Violet (Aquatic Toxicity)
- Gram Iodine (Aquatic Toxicity)
- Acetone (Ignitable, Damage to Organs)
- Gram Safranin (Contains ethanol, methanol *F003 listed* and flammable)
- Sulfuric Acid (corrosive)
- Hydrochloric Acid (corrosive)



Hazard Characteristics for Common Staining Reagents

Table 1.1 Hazard Characteristics for Common Staining Reagents

Reagent	Possible Methods	Ignitable	Corrosive	Toxic	Notes
Diff Quick Fixative	veterinary cytology	X		X	Flammable and toxic
Crystal Violet	microbiology; fingerprint analysis			X	Very toxic to aquatic life; long-lasting effect to environment
Eosin Y	histology	X		X	Toxic to aquatic life
Coomassie Blue	gel electrophoresis			X	Toxic to aquatic life
Ethidium Bromide	gel electrophoresis			X	Acutely toxic
Iodine	microbiology			X	Very toxic to aquatic life
Malachite Green	microbiology			X	Toxic to aquatic life; long-lasting effect to environment
Methylene Blue	various			X	Toxic to aquatic life
Ethanol	various	X			
Isopropanol	various	X			
Methanol	various	X			
Acetone	various	X			
Sulfuric Acid	various		X		
Hydrochloric Acid	various		X		

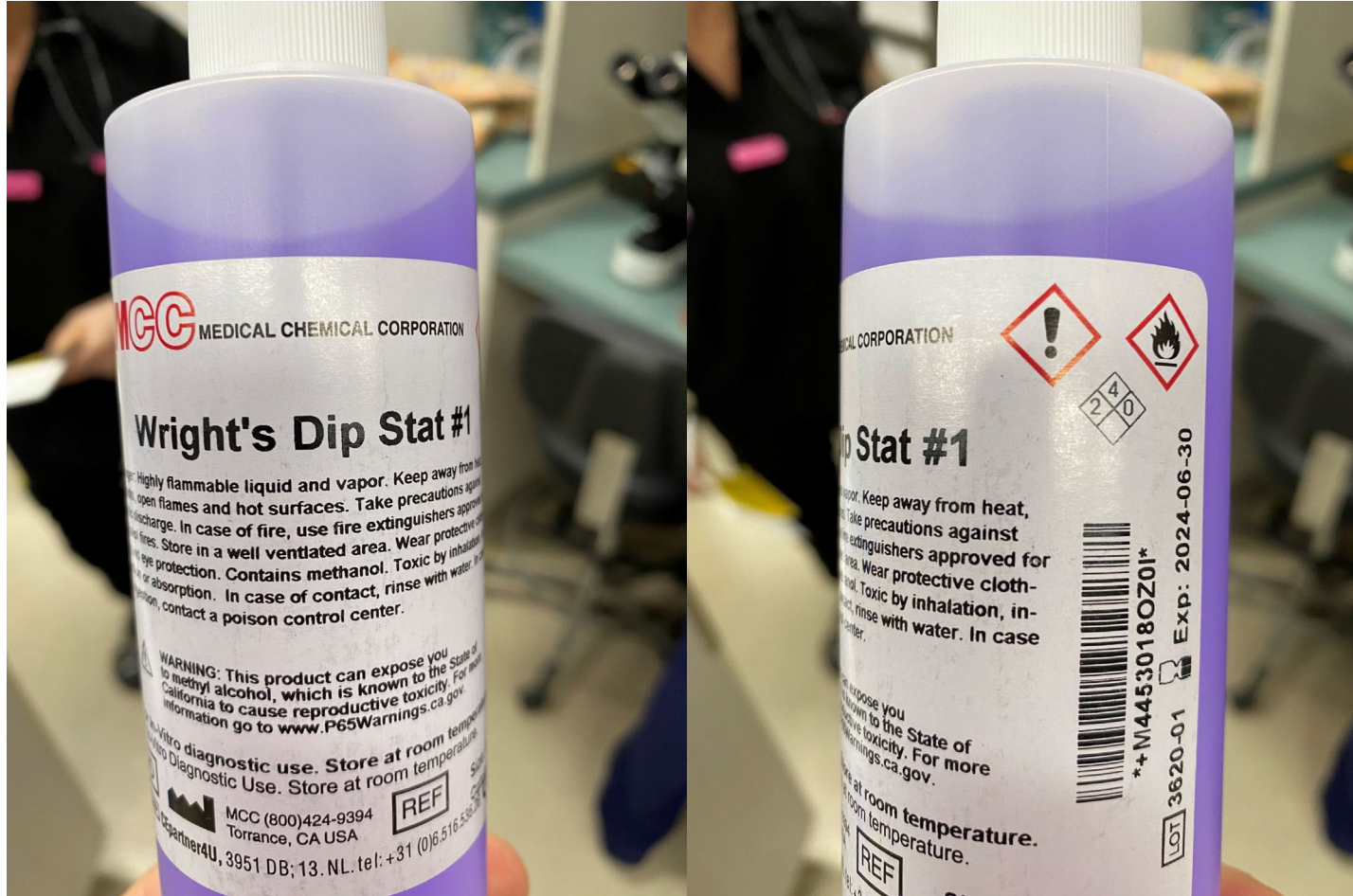


Gram Staining Kits:



- Crystal Violet
 - Flammable; toxic
- Stabilized Iodine
 - Irritant
- Decolorizer
 - Flammable; toxic
- Safranin
 - Flammable; toxic

Wright's Dip Stat #1



- Solution is highly flammable.
- Solution is an irritant
- Solution contains methanol and is toxic

Staining Stations/ Automatic Stainers



- Typical set ups are located by sink.
- Staining usually done on mats covered with paper napkins to absorb spills or directly in a sink.



- Automatic stainers contain solutions and have a receptacle to store stain waste until ready for disposal.

Safety Data Sheet: Gram Staining Iodine



SECTION 1: Identification of the substance/mixture and of the supplier

Product name: Iodine, Gram Stain Solution

Manufacturer/Supplier Trade name:

Manufacturer/Supplier Article number: S25941

Recommended uses of the product and restrictions on use:

Manufacturer Details:

AquaPhoenix Scientific, Inc
9 Barnhart Drive
Hanover, PA 17331
(717) 632-1291

Supplier Details:

Fisher Science Education
6771 Silver Crest Road, Nazareth, PA 18064
(724)517-1954

Emergency telephone number:

Fisher Science Education
Emergency Telephone No.: 800-535-5053

SECTION 2: Hazards identification

Classification of the substance or mixture:



Irritant

Skin irritation, category 2
Eye irritation, category 2A

Skin Irritation, Category 2.

Eye Irritation, Category 2.

Signal word: Warning

Hazard statements:

Causes serious eye irritation.
Causes skin irritation.

Safety Data Sheet: Crystal Violet



1. Identification

Product Name	Crystal Violet (Certified Biological Stain)
Cat No. :	C581-25; C581-100
CAS No	548-62-9
Synonyms	Gentian Violet; Basic Violet 3
Recommended Use	Laboratory chemicals.
Uses advised against	Food, drug, pesticide or biocidal product use.

Details of the supplier of the safety data sheet

Company

Fisher Scientific Company
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Emergency Telephone Number	CHEMTREC®, Inside the USA: 800-424-9300 CHEMTREC®, Outside the USA: 001-703-527-3887
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2. Hazard(s) identification

Classification

This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Acute oral toxicity	Category 4
Serious Eye Damage/Eye Irritation	Category 1
Carcinogenicity	Category 2

Label Elements

Signal Word

Danger

Hazard Statements

Harmful if swallowed
Causes serious eye damage
Suspected of causing cancer

Safety Data Sheet: Acetone-Alcohol

1.1. Identification

Product form : Mixtures
Product name : Acetone-Alcohol, 1:1, Decolorizer
Product code : LC10440

1.2. Recommended use and restrictions on use

Use of the substance/mixture : For laboratory and manufacturing use only.
Recommended use : Laboratory chemicals
Restrictions on use : Not for food, drug or household use

1.3. Supplier

LabChem, Inc.
1010 Jackson's Pointe Ct.
Zelienople, PA 16063 - USA
T 412-826-5230 - F 724-473-0647
info@labchem.com - www.labchem.com

1.4. Emergency telephone number

Emergency number : CHEMTREC: 1-800-424-9300 or +1-703-741-5970

SECTION 2: Hazard(s) identification

2.1. Classification of the substance or mixture

GHS US classification

Flammable liquids Category 2	H225	Highly flammable liquid and vapor
Serious eye damage/eye irritation Category 2	H319	Causes serious eye irritation
Reproductive toxicity Category 2	H361	Suspected of damaging the unborn child.
Specific target organ toxicity (single exposure) Category 1	H370	Causes damage to organs (central nervous system, optic nerve)
Specific target organ toxicity (single exposure) Category 3	H336	May cause drowsiness or dizziness

Full text of H statements : see section 16

2.2. GHS Label elements, including precautionary statements

GHS US labeling

Hazard pictograms (GHS US) :



Signal word (GHS US) :

Danger

Hazard statements (GHS US) :

H225 - Highly flammable liquid and vapor
H319 - Causes serious eye irritation
H336 - May cause drowsiness or dizziness
H361 - Suspected of damaging the unborn child.
H370 - Causes damage to organs (central nervous system, optic nerve)



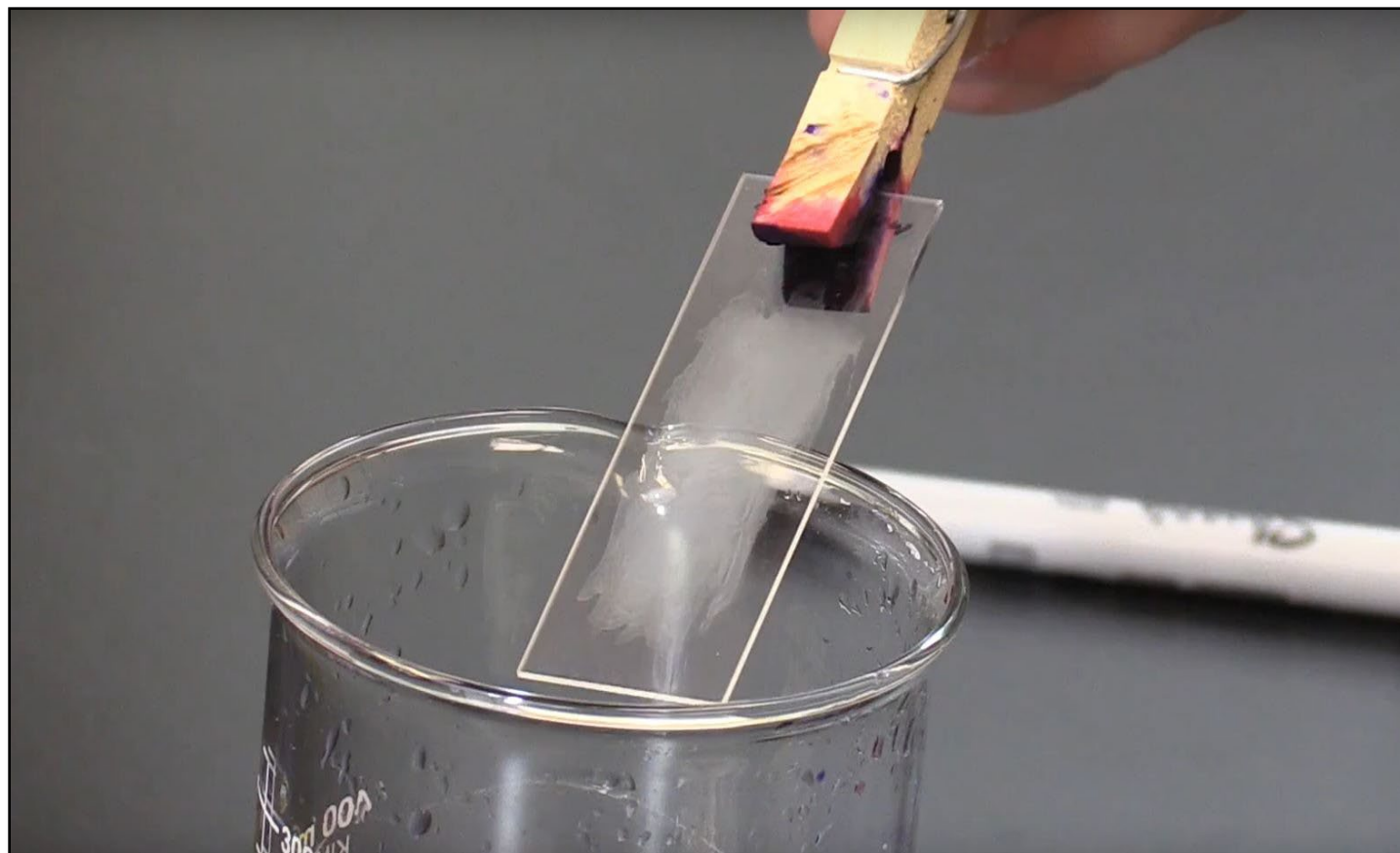
Why is staining waste hazardous?

- Reagents used in staining procedures must be managed as hazardous waste if any of the following characteristics apply:
 - ✓ Ignitable: flashpoint 140°F or 60°C
 - ✓ Corrosive: pH ≤ 2 or ≥ 12.5
 - ✓ Toxic: as defined in CCR 66261.24
 - ✓ Reactive: wastes unstable under normal conditions; or can cause explosions/release toxic fumes, gases, or vapors when heated, compressed or mixed with water
 - ✓ Toxic to aquatic life: as determined by a 96-hour fish bioassay test
 - ✓ Spent solvents may fail toxicity testing as a RCRA Hazardous Waste.



THE GRAM STAINING PROCEDURE

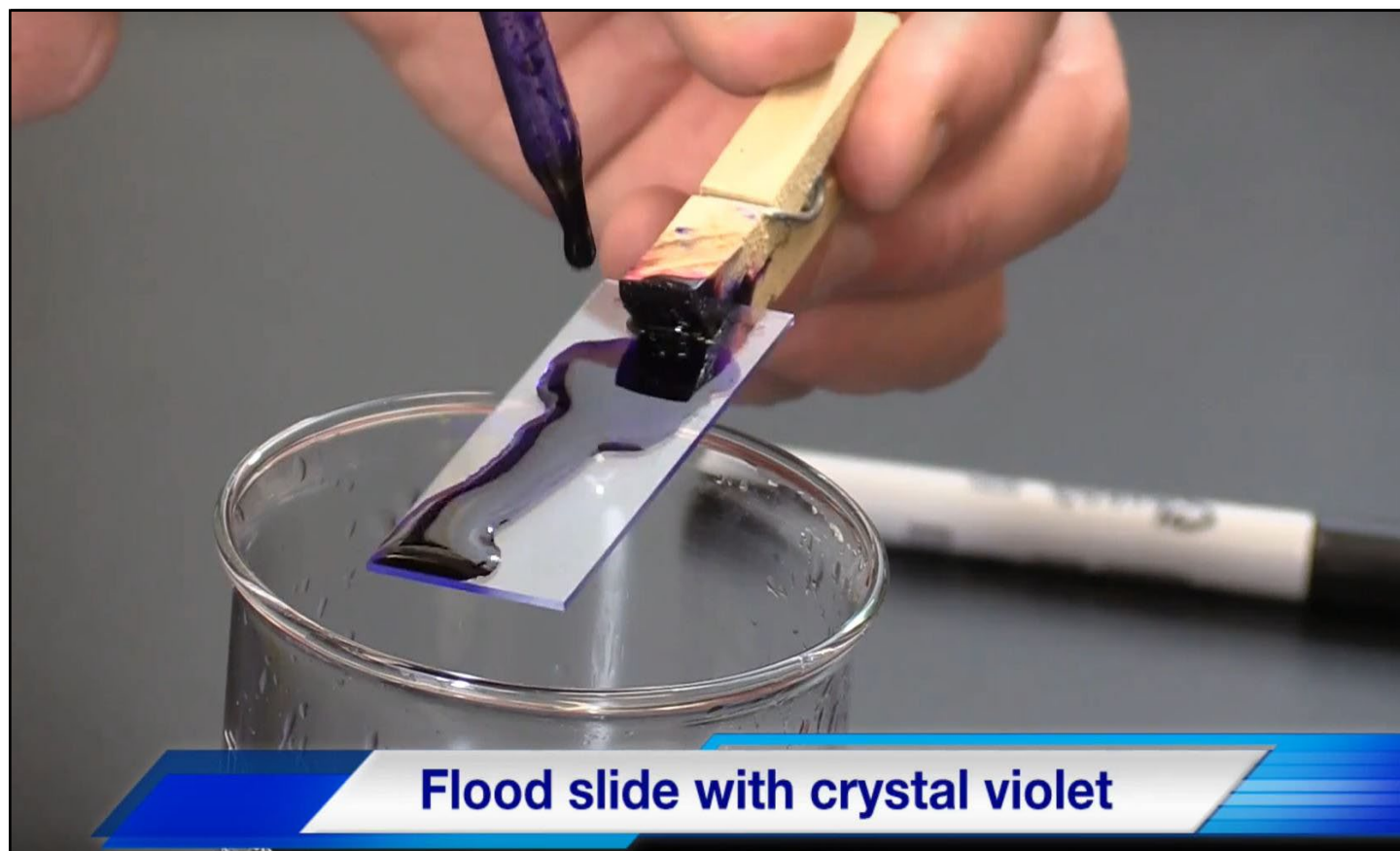
Step 1: Bacterial Smear



THE GRAM STAINING PROCEDURE



Step 2: Add Crystal Violet



Flood slide with crystal violet

THE GRAM STAINING PROCEDURE

Step 3: Water Rinse



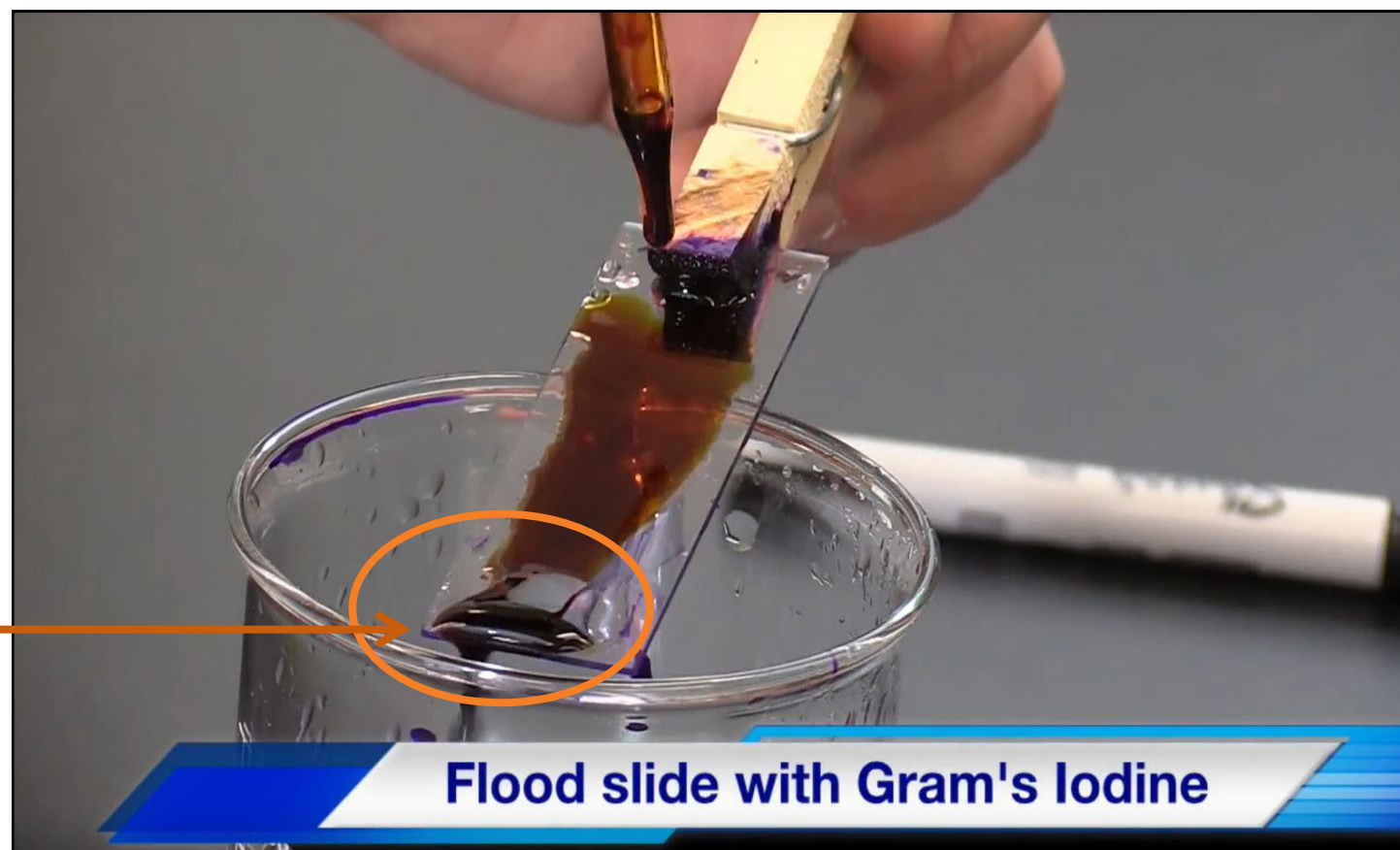
THE GRAM STAINING PROCEDURE



Step 4: Gram Iodine

Point of Generation

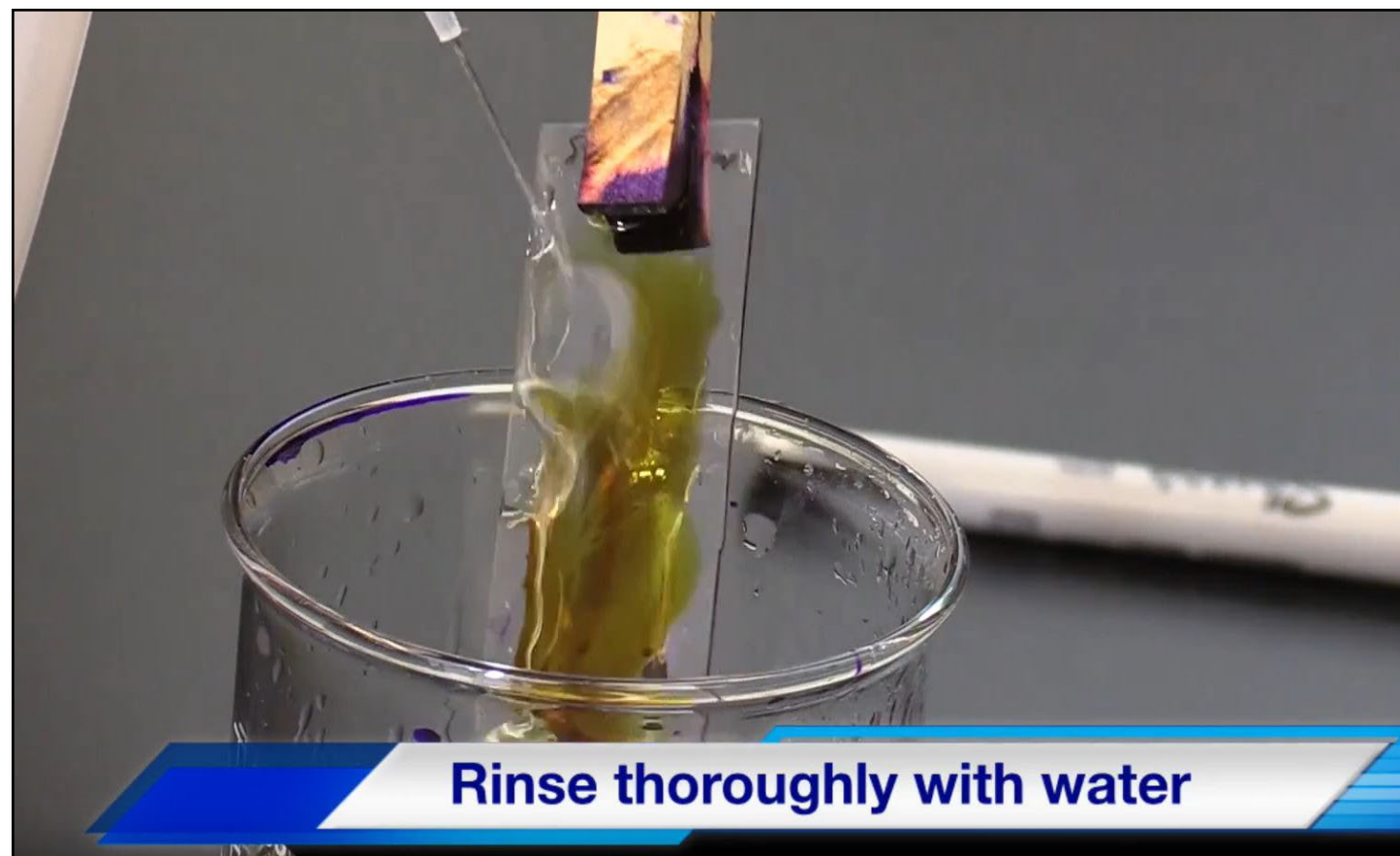
Sampling Point



Flood slide with Gram's Iodine

THE GRAM STAINING PROCEDURE

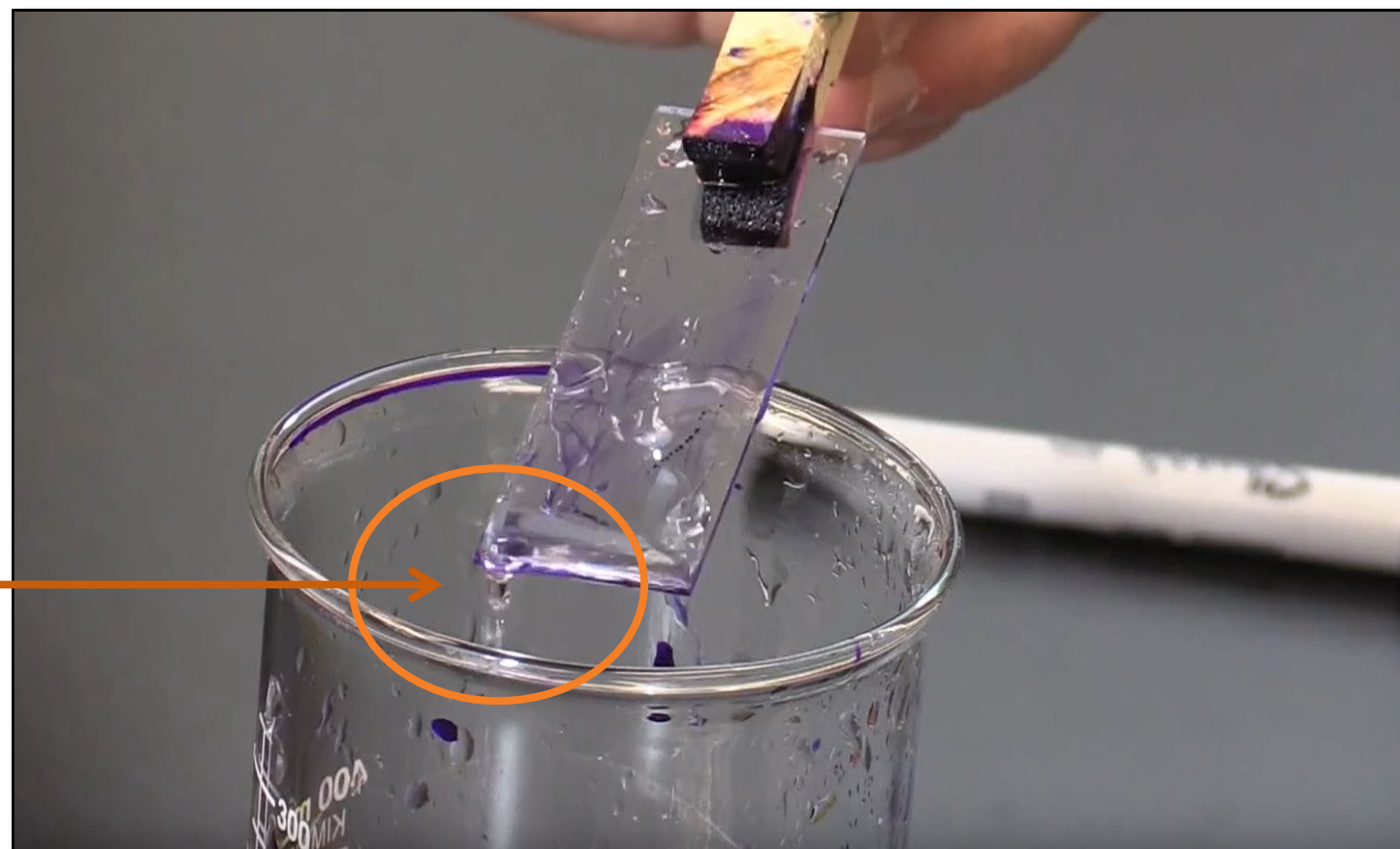
Step 5: Water Rinse



THE GRAM STAINING PROCEDURE



Step 6: Decolorize with Ethanol/Acetone



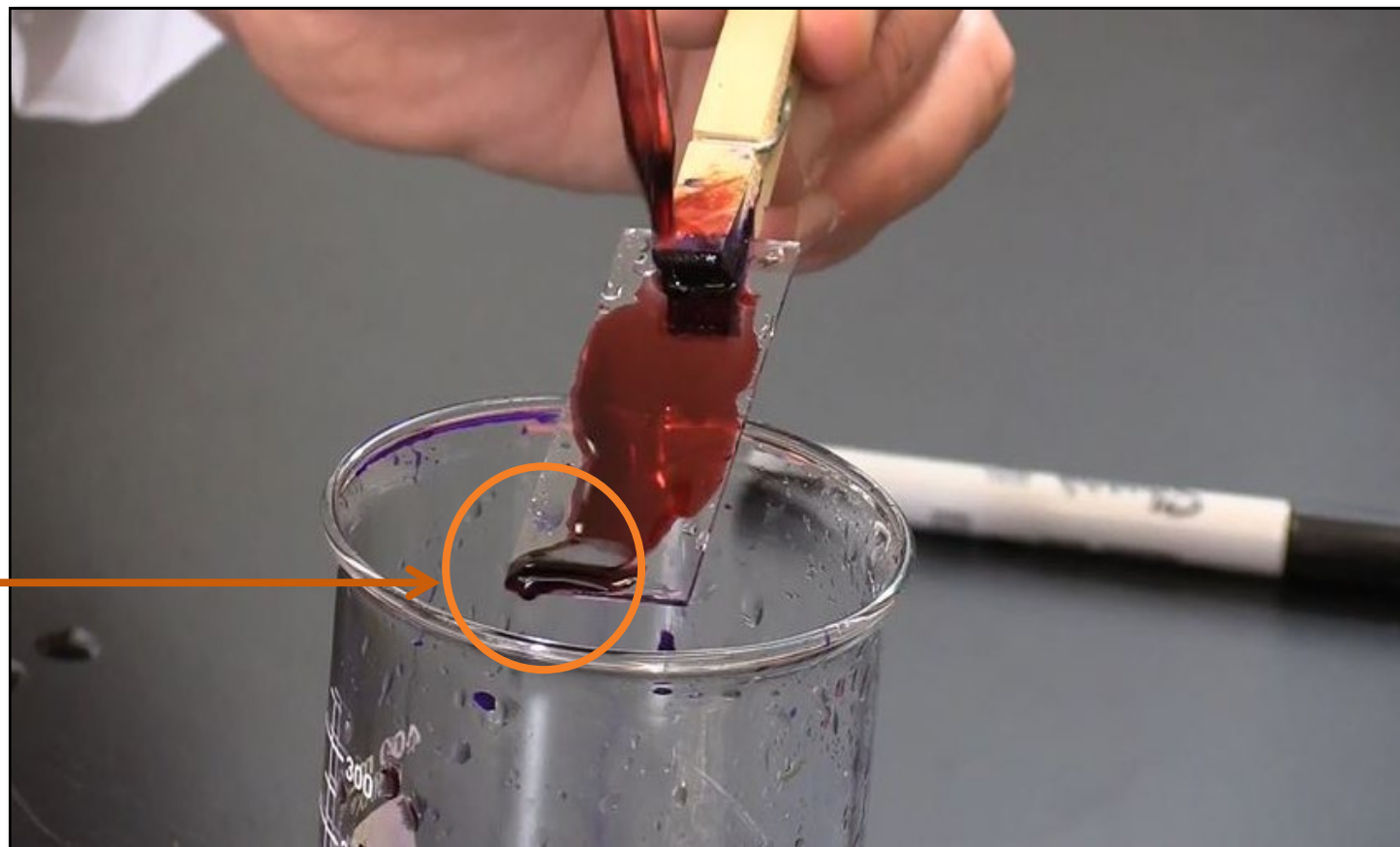
Point of Generation

Sampling Point

THE GRAM STAINING PROCEDURE



Step 7: Safranin

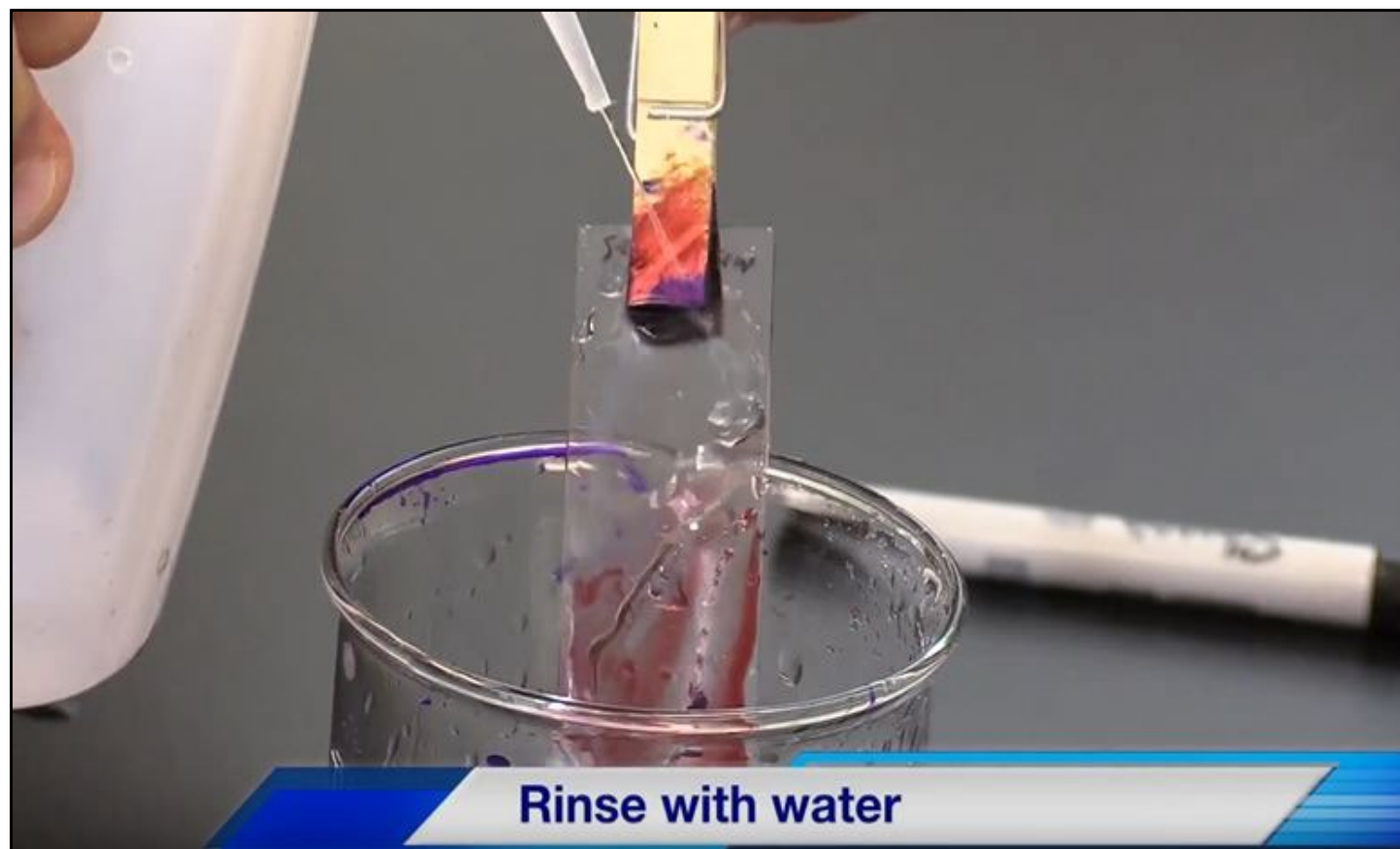


Point of Generation

Sampling Point

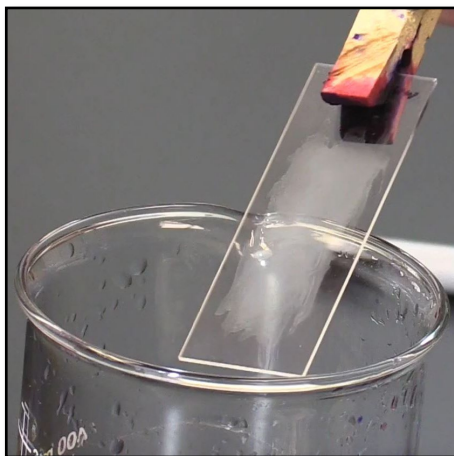
THE GRAM STAINING PROCEDURE

Step 8: Water Rinse

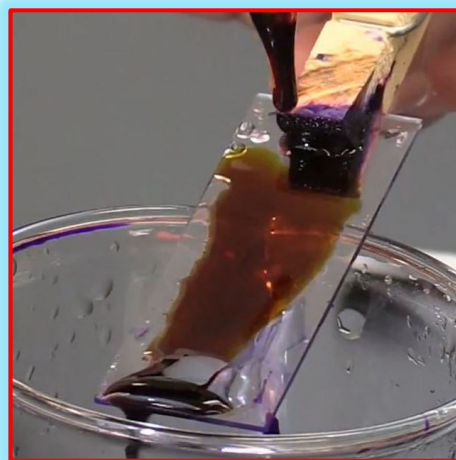


GRAM STAINING

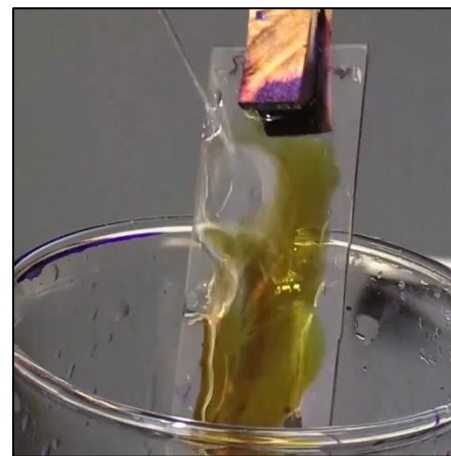
Hazardous Waste Management & Points of Generation



Bacterial Smear



Staining



Water Rinsing



Decolorizing w/ Acetone
or Alcohol

****Red indicates high possibility of release of hazardous waste at the point of generation**

Evidence of Staining



Crystal violet staining is apparent and difficult to remove. Could be clear evidence of improper waste management.



Evidence of Staining



Evidence of: Crystal violet staining, improper waste management, lack of personnel training, facility may not have waste minimization protocols in place.

How should stain waste be managed?

- When hazardous, stain waste must be stored in closed containers.
- Store in closed containers except when adding/removing waste.
- Clearly label the container with:
 - The words, "Hazardous Waste"
 - Date waste accumulation began
 - Waste composition, physical state, and hazardous properties
 - Name and address of the facility
- Schedule to have hazardous waste transported to a TSDf by a registered transporter, within required time frame; dependent on generator status.
- Other generator requirements apply.
- **Hazardous staining waste must not be disposed of to the sewer.**
- **Staining rinsate may also be hazardous and requires a hazardous waste determination at the point of generation.**



Example Questions for Lab & EHS Staff

- Does personnel on site conduct staining?
- What is your staining process? Manual or automatic?
- When do you change out the reagents?
- How and where do you dispose of the reagents?
- How much (volume) is disposed?



Best Management Practices

- Store SOP at staining station which also addresses waste minimization.
- Provide specific training to reduce amounts of reagents used.
- Ensure sinks are plumbed to appropriate container; must maintain closed in a manner that prevents unplanned release of contents.
- Use automatic stainers to automate process and reduce waste.
- Maintain waste collection container in immediate vicinity of staining station to collect staining wastes (satellite accumulation).
- Determine if rinsate waste is hazardous or not by an approved method --knowledge of process, testing, industry standards.



Plumbing at Sink



Bad: Open container, not appropriately containerized, spill potential is high, potential

VS



Good: Sealed container, minimal spill potential, easy removal and replacement.

Satellite Accumulation

- Up to one year of storage if less than or equal to 55 gallons of waste stream/process.
- Once waste reaches 55 gallons, it must be moved to central accumulation area within 3 days.
- Two dates on HW label: first date indicates when container began accumulating waste; second date indicates when it reached the satellite accumulation limit.



Possible violations for mismanagement of stain waste:

- Failure to make a proper waste determination
 - Typically, Class I (NOV)
- Failure to properly dispose of hazardous waste at an authorized facility.
 - Class I (NOV)
- Failure to ensure employees are trained on proper waste handling procedures
 - Class II, at minimum
- Failure to report a hazardous waste in CERS.
 - Class II, at minimum



How should stain waste be reported in CERS?



- Common name: Stain Waste OR Gram Staining Waste OR Spent Reagents
- Physical state: liquid
- Material Type: waste
- Waste code: dependent on waste classification
- Storage temperature: ambient
- Storage pressure: ambient



Resources:

- **HMD website:** <https://www.sandiegocounty.gov/content/sdc/deh/hazmat/hazwaste/hazwastedetermination.html>
- **HMD Staining Guidance:** [https://www.sandiegocounty.gov/content/dam/sdc/deh/hmd/pdf/HMG-3023%20\(10-22\)%20-%20Staining%20Waste%20Guidance.pdf](https://www.sandiegocounty.gov/content/dam/sdc/deh/hmd/pdf/HMG-3023%20(10-22)%20-%20Staining%20Waste%20Guidance.pdf)
- **California Hazardous Waste Regulations:** <https://dtsc.ca.gov/title22/>
- **Waste reduction guidance:** <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3577235/>
- **How to manage Hazardous Waste:** <https://dtsc.ca.gov/managing-hazardous-waste/>



Questions?

