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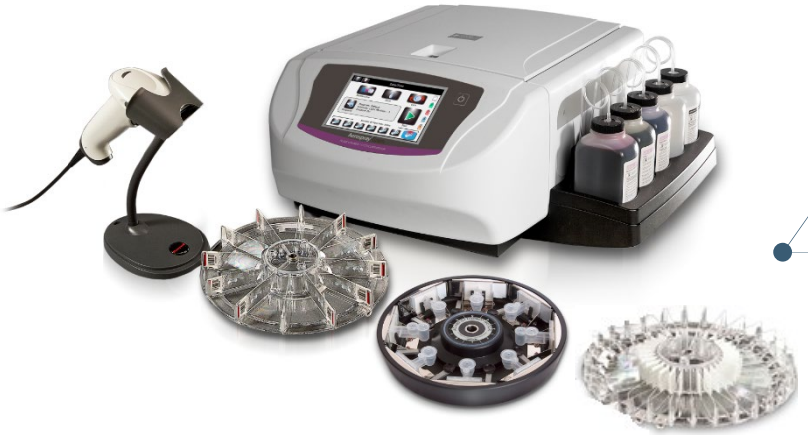
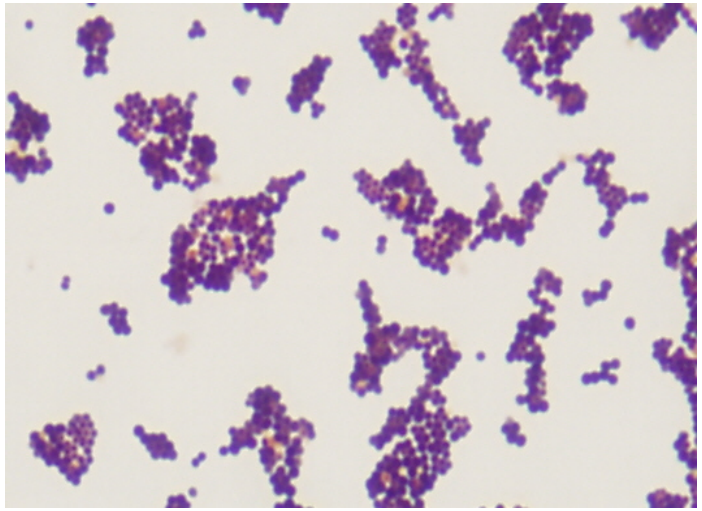


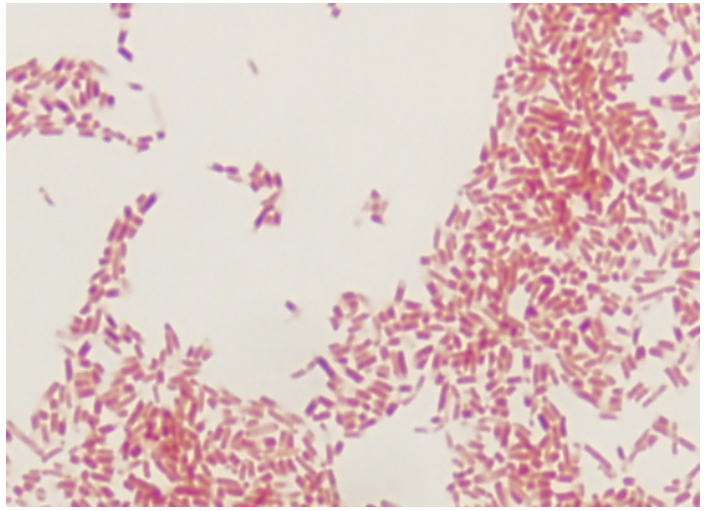
Aerospray® Gram Automated Slide Stainer/Cytocentrifuge (Model 7322)

Applications Training

Gram Stain Overview

- Developed ~125 years ago by Hans Christian Gram.
- Still widely used throughout microbiology.
- Stains bacteria, parasites, yeast (fungi), etc.
- Differentiates bacteria into two large groups, which is used as a first level classification.
  - Gram Positive (blue/purple)
  - Gram Negative (pink/red)
- Performed on body fluids or biopsies when infection is suspected.

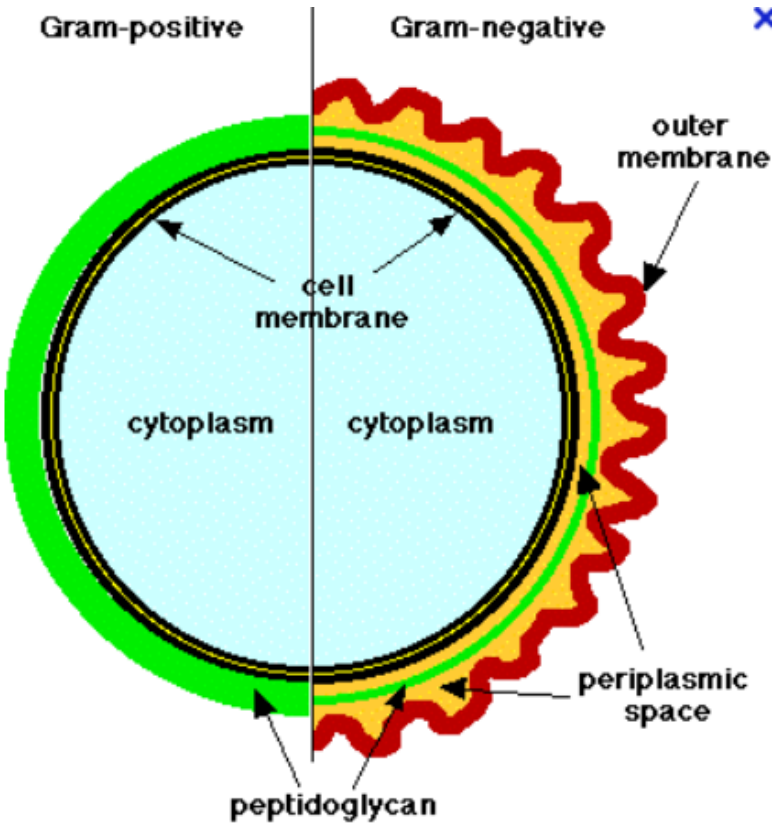




Preparing a Gram Stain

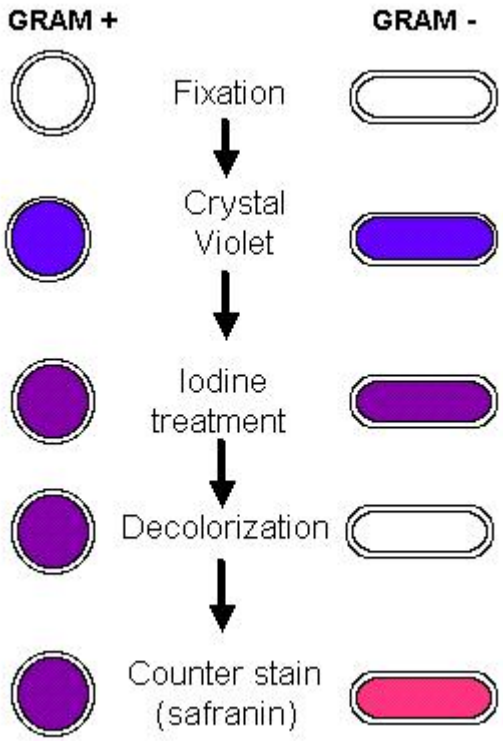
- Cell concentration
  - Conventional centrifugation or
  - Cytocentrifugation (Cytopro Rotor)
- Fix Slides
  - Heat
  - Alcohol (methanol or ethanol)
- Apply Crystal Violet reagent
- Apply Mordant (Iodine), precipitates with CV in the cells
- Wash out precipitate with alcohol. CV precipitate washes out slower in Gram positive bacteria because of the thick peptidoglycan cell membrane.
- Counter stain Gram negative bacteria pink.

It's all in the cell wall!



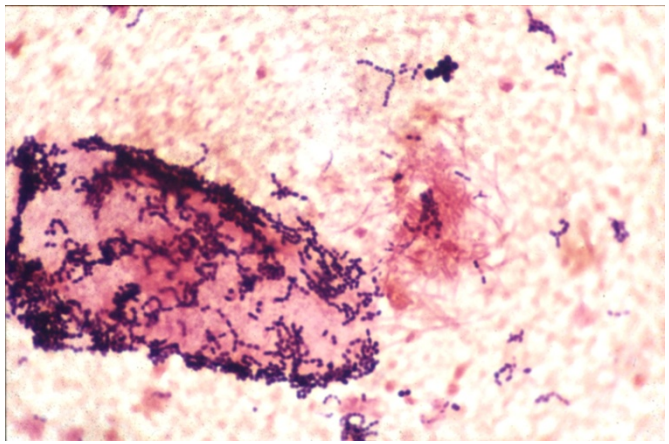
Preparing a Gram Stain

- Fixation (optional) – Methanol (PUMP E)
- Primary Stain – Crystal Violet (PUMP C)
- Wash – H_2O (PUMP D)
- Mordant – Iodine (PUMP B)
- Wash – H_2O (PUMP D)
- Decolorization/Counterstain (PUMP A)
- Wash – H_2O (PUMP D)
- Spin Dry



When to Perform a Gram Stain?

- Preliminary classification of causative agent to initiate a fast presumptive diagnosis and treatment
- Antibiotic / Antimycotic treatment monitoring
- Pre-screening of sputum samples
- Identification of bacteria in sweat (Johnson& Johnson)
- Screening of water lines for beverage suppliers (Coca Cola, Gatorade)



Contaminated sputum mixed flora (no predominance).

Advantages of Cytopro Rotor with Gram Stainer

- Concentrating samples with Cytopro is optional, but recommended when possible.
- Concentration of body fluids for superior sensitivity
- Uniform thin slide for reproducible staining result
- Fast (save 20 minutes/sample)

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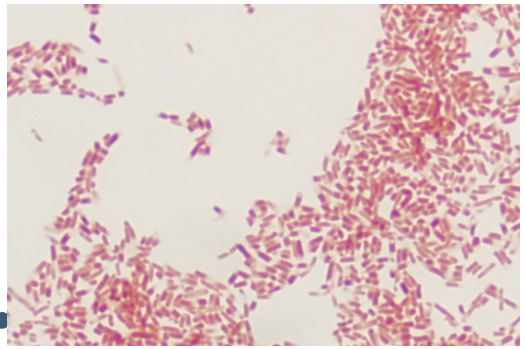
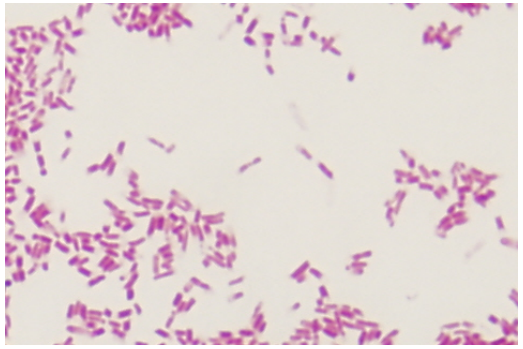
Gram Staining Reagents

- Reagent A: Decolorizer/Counterstain
  - Decolorizer and Counterstain is added together. Options:
    - Safraine dye: mostly used in US and Asia.
      - ✓ Safranine Dye with Methanol and Isopropanol (SS-041A)
      - ✓ Safranine Dye with Isopropanol and Acetone (SS-041AA) – EBS preferred.
    - Fuchsin dye: mostly used in Europe.
      - ✓ Fuchsin Dye with Methanol and Isopropanol (SS-041AF)
      - ✓ Fuchsin Dye with Isopropanol and Acetone (SS-041AAF)
- Reagent B: Mordant- Iodine
  - 6 month shelf life for ready to use stain (SS-041B)
  - 8 month shelf life for concentrate (SS-141B)
- Reagent C: Primary stain- Crystal violet

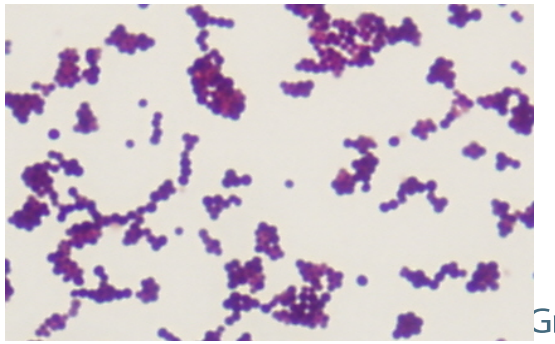
E.Coli -safranin



E.Coli -fuchsin



S.aureus-fuchsin



Gram Staining Reagents

- There are four different decolorizer/counterstain reagents that can be used.

Decolorizer	Description
SS-041A– Safranin	• Including 60% Isopropanol, 40% Methanol • More orange G-
SS-041AA – Acetone with Safranin	• Including 75% Isopropanol, 25% Acetone • Gives stronger gram positive result. • EBS recommended
SS-041AF - Fuchsin	• Including 60% Isopropanol, 40% Methanol • Used mainly in Europe • More purple G-
SS-041AAF – Acetone with Fuchsin	• Including 75% Isopropanol, 25% Acetone •Gives stronger gram positive results.

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7322 Prepared & Concentrate Reagents

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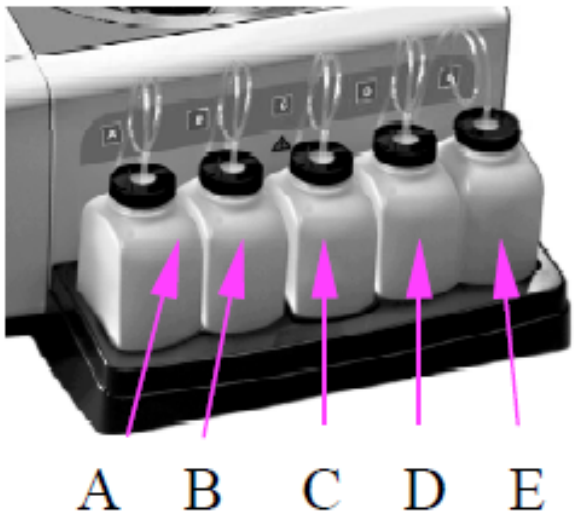
Gram Staining Reagents

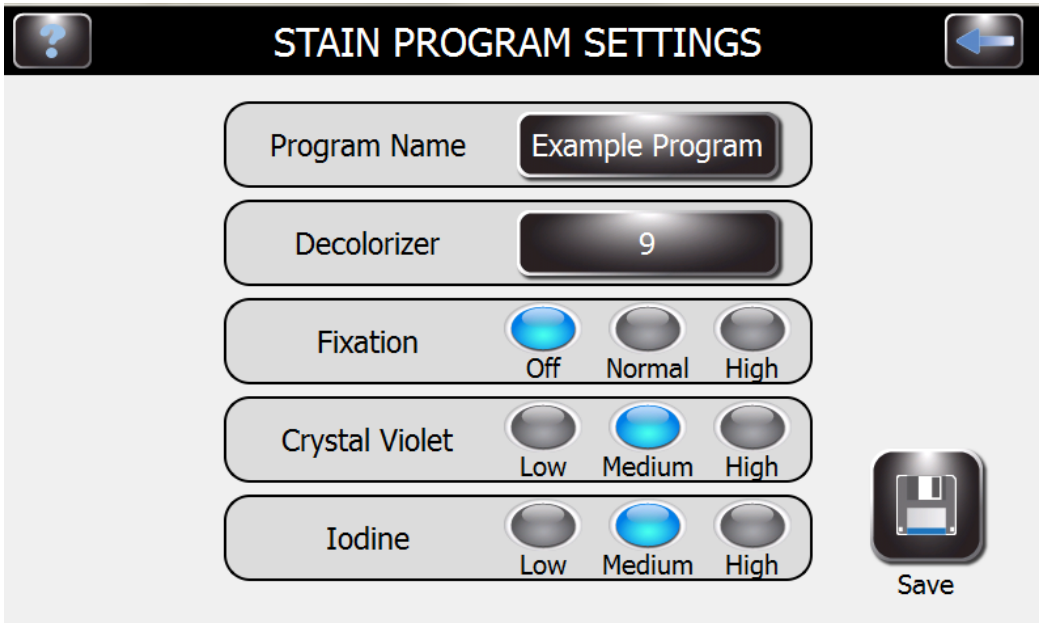


1 Place the reagent bottles front to back in the following order:

(A) Decolorizer with Counterstain
(B) Iodine
(C) Crystal Violet
(D) Deionized Water
(E) Methanol or Ethanol

Programming Flexibility

- 9 decolorizer settings.
- Crystal violet (CV) and Iodine (I2) adjustments
  - High settings of CV and I2 will make Gram positive bacteria stronger (more positive)



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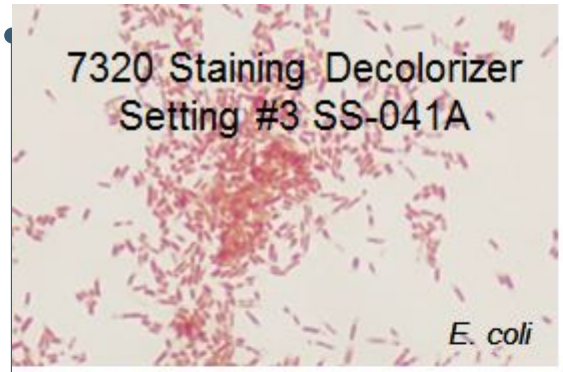
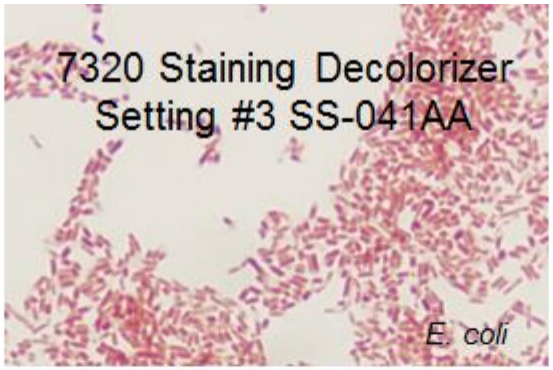
Programming Flexibility

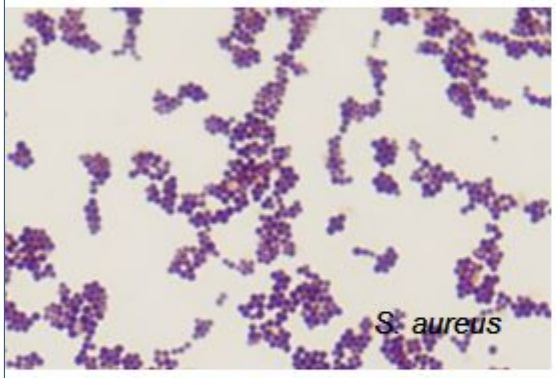
- Most customers will find a setting that works well for all of their samples.

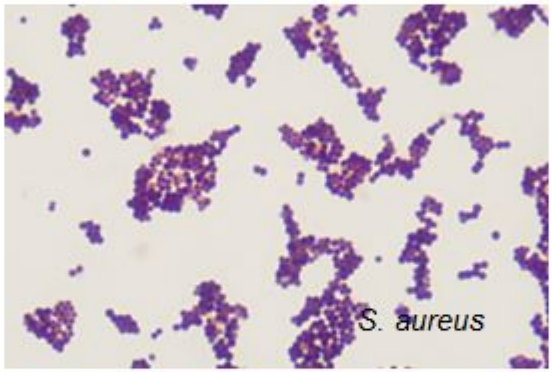
Decolorizer Setting	Thickness	Suggested Specimen
1	Very thin Smears	Old culture(s)
2	Thin Smears	CSF, urine, peritoneal, vaginal, wounds, bronchial washes, etc.
3	Thin and medium smears	Vaginal, sputum, bronchial washed, CSF, urine, wounds, etc.
4		
5	Medium and Thick Smears	Sputum, bronchial washes, blood cultures, etc.
6		
7	Thick smears	Sputum, bronchial washes, blood cultures, etc.
8		
9	Very Thick Smears	Stools, blood cultures, etc.

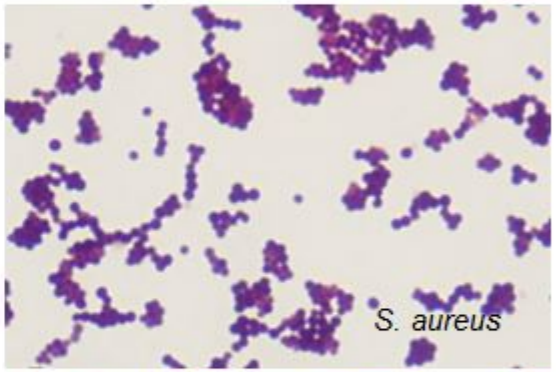
7322 Decolorizer/ Counterstain Comparison

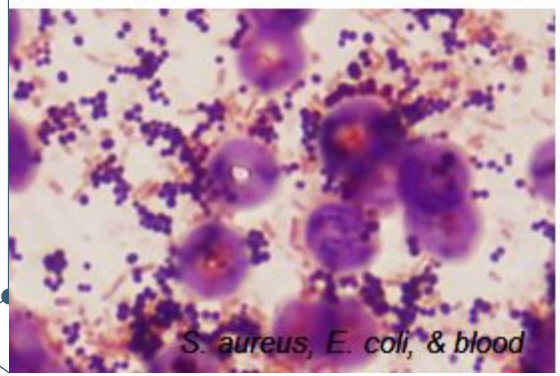


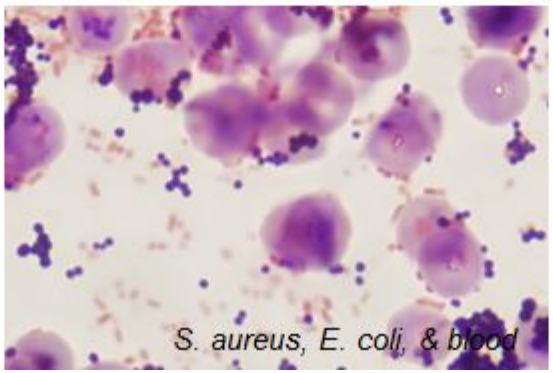


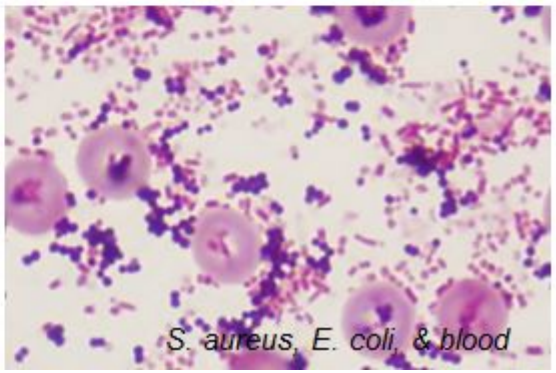












Quality Control Management

ELITechGroup Gram Control slides SS-250 (50 slides)

- For quality control management
- Gram positive: S.aureus – Gram negative: E.coli

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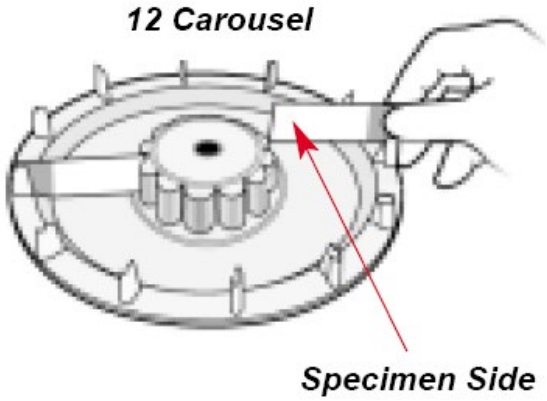
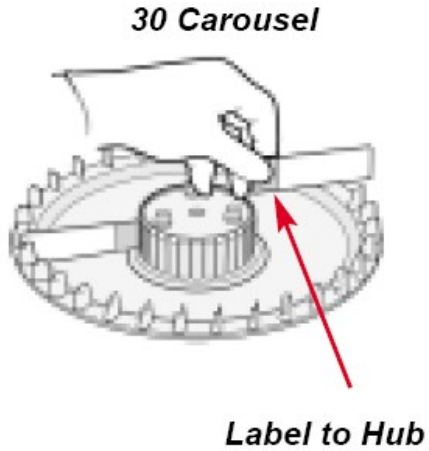
Advantages of Gram Stainer

- No cross contamination
- Fast, easy, CLEAN
- Consistent staining
- Economical, more slides with less stain.
  - Much less stain waste generated.
- 12 slide or 30 slide carousel
- Stainer/Cytocentrifuge combination
- Flexibility
  - Nine different decolorizer settings
  - Increased Crystal Violet and/or Iodine spray
  - Optional fixation
- Minimal maintenance





7322 Disadvantages

- **Over/under decolorization**
  - Over decolorization: Gram positive organisms stain pink
  - Under decolorization: Gram negative organisms stain purple
- **Nozzle maintenance: Iodine (B line) needs to be checked or cleaned once a week.**
- **Bowl fouling with Crystal Violet and Iodine precipitate**
- **Short Expiration date of Iodine (six months)**
- **Monthly B-line flush**

Overcoming the 7322 Disadvantages

- **Over/under decolorization**
  - Decolorizer setting of 3 or 4 are comparable to hand staining with 30 or 40 seconds of stationary decolorizer treatment.
  - It is well known that gram positive cultures, such as Bacillus sp. And Streptococcus sp., can easily be over-decolorized, especially when cultures are old or stressed.
  - The 7322 is more sensitive to these cultures than hand staining. (Remind customers to use fresh cultures (log phase) when these specimens are used as controls)
  - Samples at the upper and lower edges of the slide may over-decolorize because specimens do not get best stain coverage.

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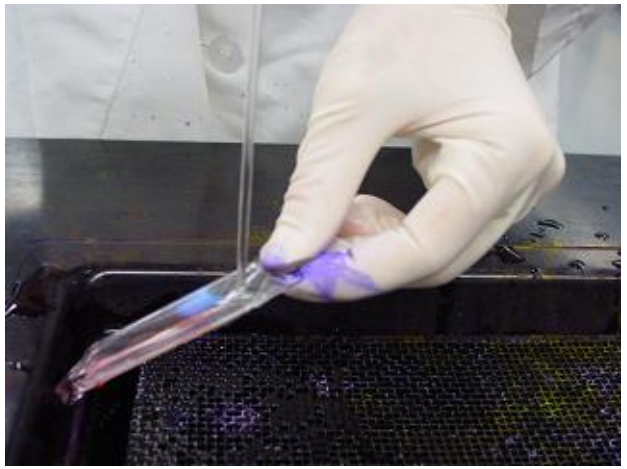
Competition: Hand Stains

- Inconsistent results
- More reagent consumption
- Time consuming (separate decolorizer/counterstain step + slides not dry)
- Contact with hazardous stain
- Not clean, messy sinks.
- No traceability and not user-friendly



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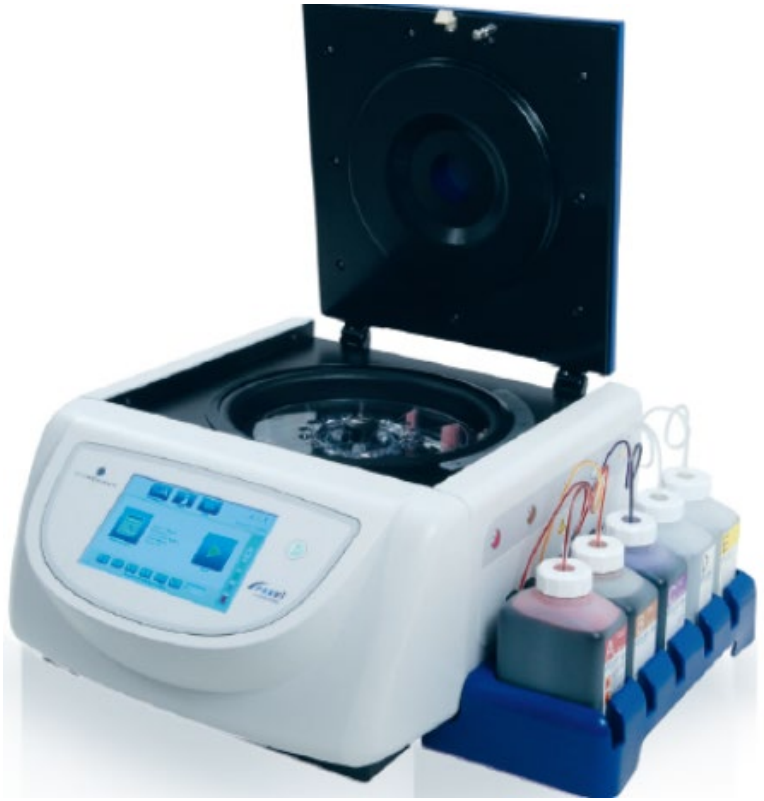
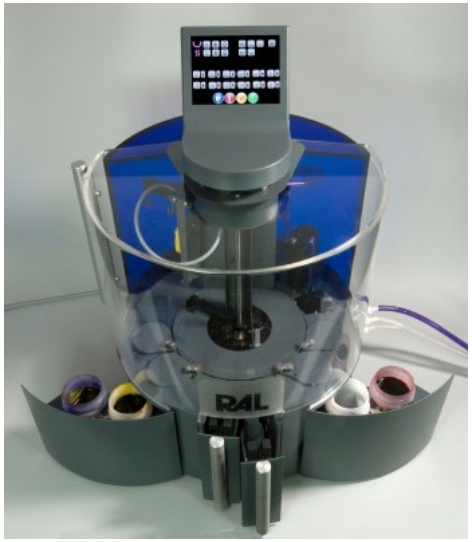
Competition: PREVI Color Gram

- EBS is manufacturer
- EBS has much more knowledge & service available.
- More expensive (device+reagents) as barcode reader, 5L reagent level and 10L waste level detection is not optional
- Samples can only be entered via barcode, not manually
- 6 nozzle starter kit, EBS 2 nozzle starter kit
- BioMérieux cannot offer full stainer range, only Gram Stainer and TB with different technology



Competition: Dip Stainers

- Dip stainers in general:
  - Cross-contamination (batch for 60 samples)
  - More reagent consumption
    - Dispose reagents or filter them is cell washoff is expected.
  - Reagents can be exposed to environment.
  - Fumes from reagents.
  - Wet slides.
  - Limited traceability.
- RAL
  - $0,50-$1,00/slide (EBS 0,03$/slide)
  - Slower TAT (15’ 40 slides – ELITechGroup 9’ for 60 slides)
  - Short shelf life of reagents once opened.
  - Reagent waste.



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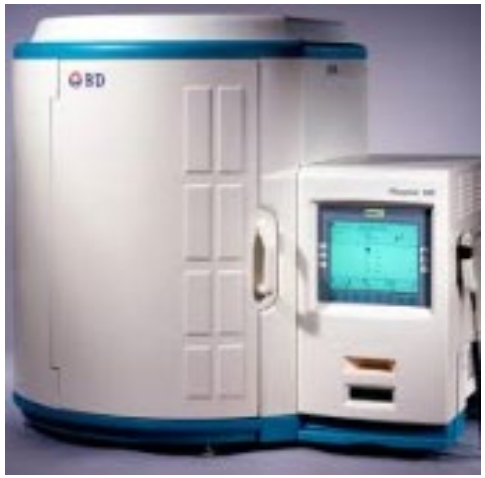
Market Development

- Support is where we can differentiate ourselves.
- Evaluate collaborations with local suppliers (BD, Siemens) to offer a more complete solution in tenders.
- Focus on labs preferring manual sample entry.
- Always promote Cytopro in parallel as a niche solution!

Market Development

- Do you also collaborate with another partner?
  - Latin American
    - Aerospray Gram + Phoenix (BD)
  - Germany
    - Aerospray Gram + WASP® (Copan)
    - Total of 102 stainer in Germany, some placed along with WASP.



Questions to ask during sales visit

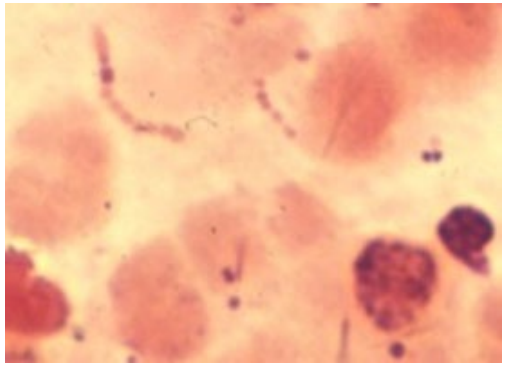
- How do you Gram stain your microbiology samples?
- What type of reagents does your lab use? (explain safranine/fuchsin, acetone or methanol)
- How many samples does your lab stain/day? (explain reagent calculation tool website – economical benefits)
- Do you sometimes encounter problems with uniformity of slides or cross-contamination? (explain Aerospray fully automated, standardized system)
- How do you treat your waste? (explain limited reagent consumption)
- Does your internal QC management or accreditation process require traceability for patients and reagents.
- How do you concentrate your body fluids? (explain Cytopro option)
- Would you be interested in a dual purpose cost-efficient staining/cytocentrifugation system?
- How is re-imbursement arranged (per test or global budget)?

Performing a Successful Demonstration

- Prospection:
  - Understand the customer's fixation protocol and preferred staining reagents.
  - Show EBS stained control slides to preliminary select preferred decolorizer setting.
  - Present Cytopro rotor for standardized sample preparation
- Demo preparation:
  - Ask customer to prepare fresh samples (max 24h) . Record 'abnormal' characteristics before staining.
  - Include SS-250 Control slides.
- Actual demo:
  - Start with Decolorizer setting 4 or setting customer preferred on stained control slide.
  - Staining control slides (SS-250), can help determine preferred Decolorizer setting.
  - For new accounts, set up stainer with safranine + acetone (SS-041AA).
  - Demo on Cytopro rotor.

Performing a Successful Demonstration

- Case study 1: Question
  - The Jyntendo Hospital in Japan evaluated the Gram stainer. They used acetone with safranine and obtained the following stain result at decolorizer setting 7.



- How does the slide look?
- What can cause the slide to look like this?
- How can we modify Aerospray Gram settings to meet the customer need?

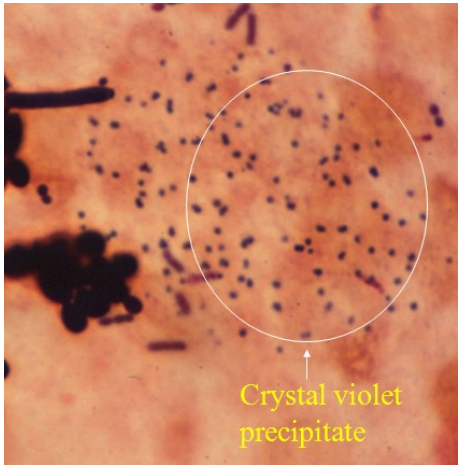
Performing a Successful Demonstration

- Case study 1: Answer
  - Over-decolorization
  - Solutions:
    - First exclude error on test operation, but all ok
      - Was all methanol removed from lines during set-up?
      - Reagents correctly prepared?
      - Nozzles clean?
      - Reagent grade acetone used?
      - Fresh sample, lag phase?
    - Decolorizer setting should be decreased to setting 3
    - In addition, Crystal Violet/Iodine can be increased to « High »

Note: G+ (Bacillus, Strep) are more sensitive to over-decolorization

Performing a Successful Demonstration

- Case study 2: Question
  - The Policia Lab in Colombia evaluated the Gram stainer. They used safranine with acetone. They observed over-decolorization and debris on slide.



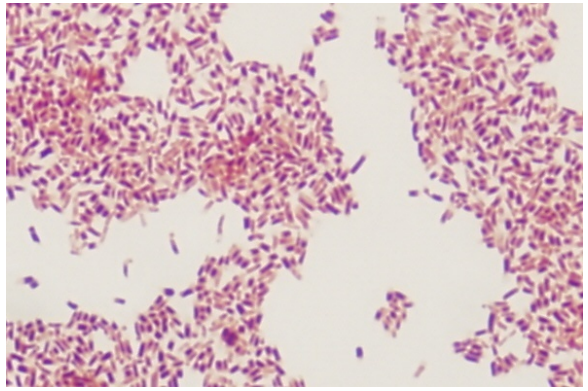
- What can cause debris to be seen on slide and how can we prevent it?

Performing a Successful Demonstration

- Case study 2: Answer
  - Causes: expired iodine used
  - Other possible causes:
    - B line (iodine) not properly cleaned (monthly flush). Iodine debris can be present on the slide.
    - D nozzles (water) not properly cleaned.
      - D line bottle should be cleaned with bleach (sodium hypochloride) one per month. If not cleaned the line can be contaminated causing debris on the slide.
    - Dirty slides used. Debris stained on the slide.
    - Fresh sample?

Performing a Successful Demonstration

- Case study 3: Question
  - The ARUP Lab in Salt Lake City Utah evaluated the Gram stainer. They observed under-decolorization on a control slide. (Purple stain on a lot of G- organisms.



- What can cause under-decolorization? How is it corrected.

Performing a Successful Demonstration

- Case study 3: Answer
  - Causes: Decolorizer setting is too low. Increase decolorizer setting number.
  - Other possible causes:
    - Check A nozzle, make sure it is spraying properly.