



# The Basics of Fuchsin

AIMS NSM 2009,  
Adelaide

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## 2009: 150<sup>th</sup> Anniversary

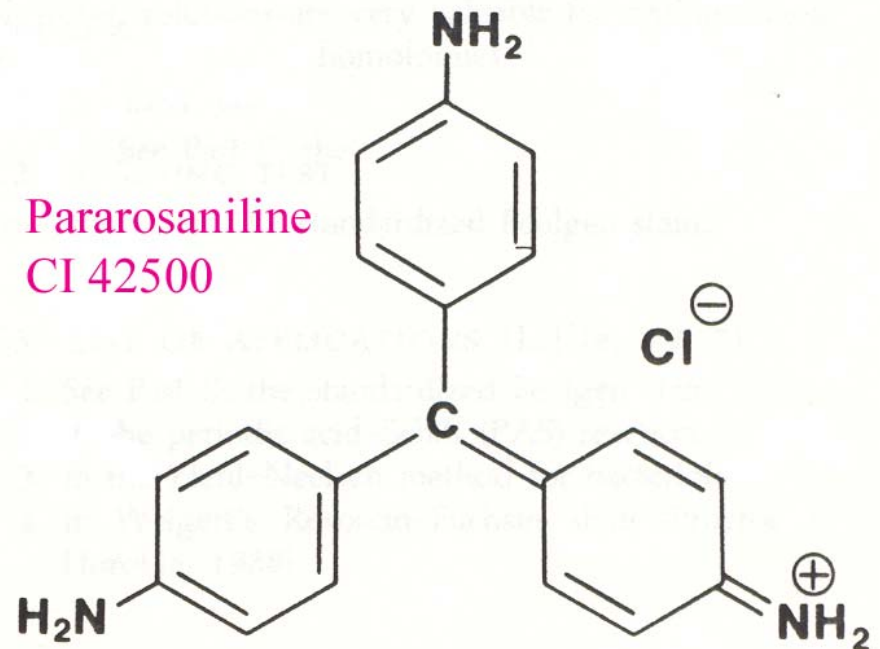
- First commercial production in 1859
  - 2<sup>nd</sup> “aniline” or synthetic dye produced
    - after aniline purple / mauveine (Perkins, 1856)
- Verguin, (France) saw commercial potential
  - joined commercial dyers “Renard frères et Franc”
  - patented the process of synthesis
  - etymology (name and colour of fuchsia flower)
- Why “aniline dye”?
  - distillation of coal tar → aniline → dye

# Some More History

- Use as biological stain (Waldeyer, 1864)
  - nuclei and nerve axons
- 1870's: distinction of acid and basic dyes
- 1877 structure determined (Fischer & Fischer)
  - subsequently, isomeric components and derivatives determined

# Basic Fuchsin: Some Chemistry...

- Aryl methane dye
  - 4 triaminotriphenylmethane homologues
    - resonance
      - $H^+$  and quinonoid group move
    - strong vibrant colours
  - Use trivial or informal names
    - Conn's Biological Stains 10<sup>th</sup> ed.
    - lower case (unless proper noun)
  - CI numbers / names
    - 3 of 4 compounds
  - cationic dye
  - chloride or acetate salts



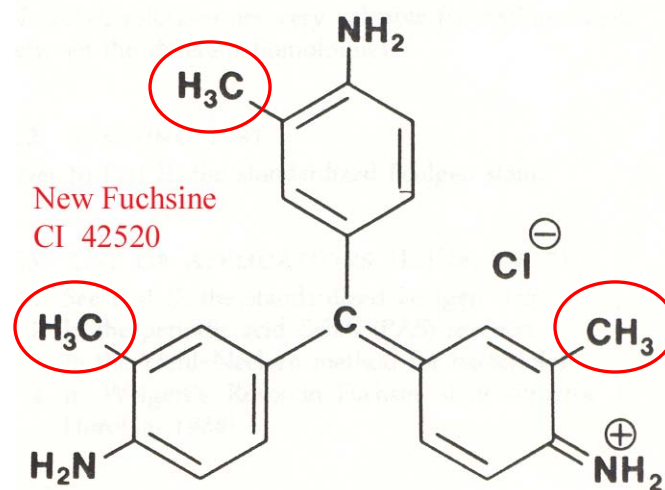
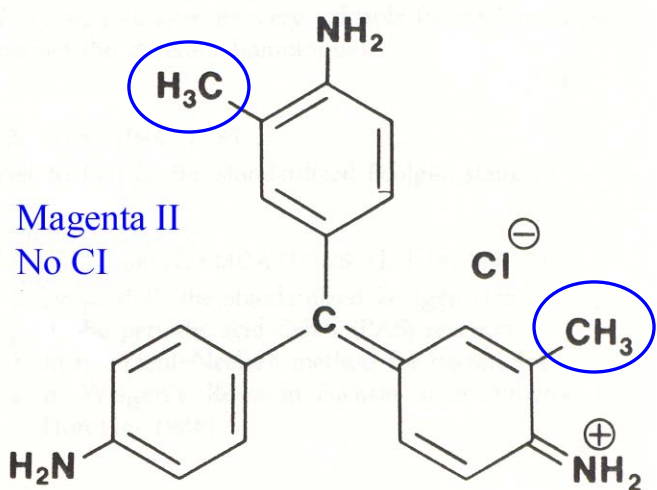
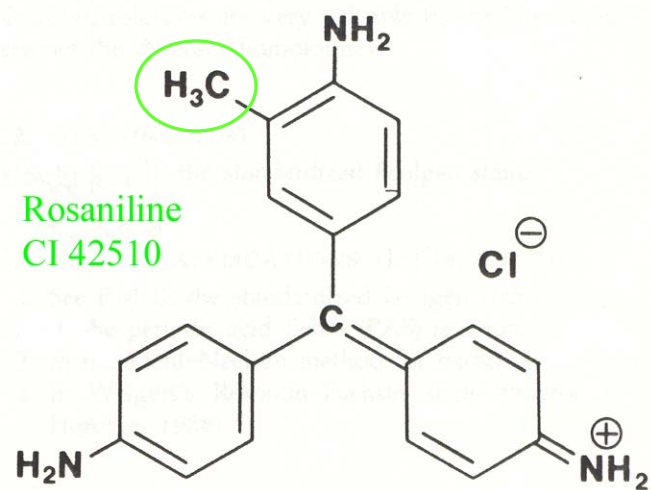
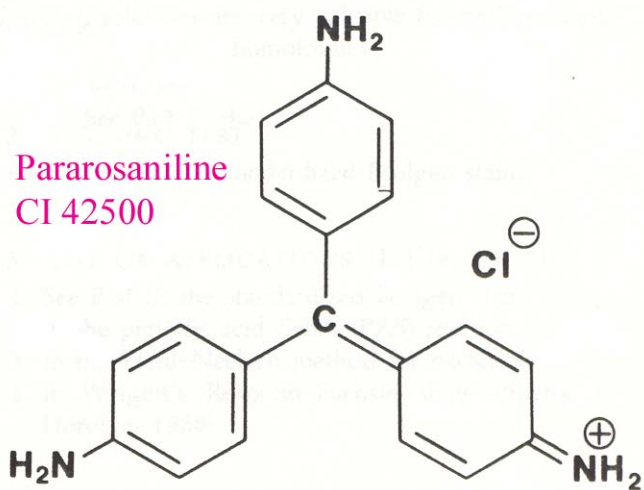
-iminium ion

# What's in a Name?

- basic fuchsin vs. basic fuchsine
  - John Kiernan
    - “.. the ending –ine is appropriate for an organic base or compound derived from an organic base.”
- Dye tends to behave more like a salt, as will not lose proton until pH approximates 11.

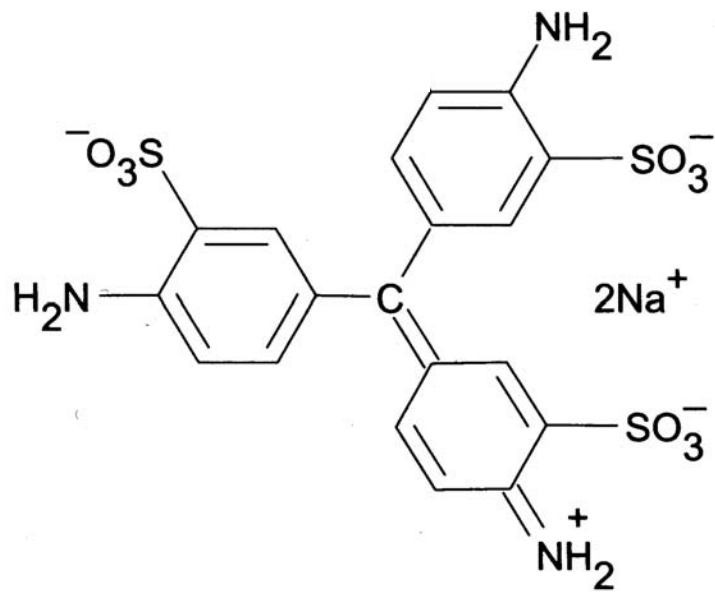
*base = proton acceptor*

# Basic fuchsine: Some Chemistry...

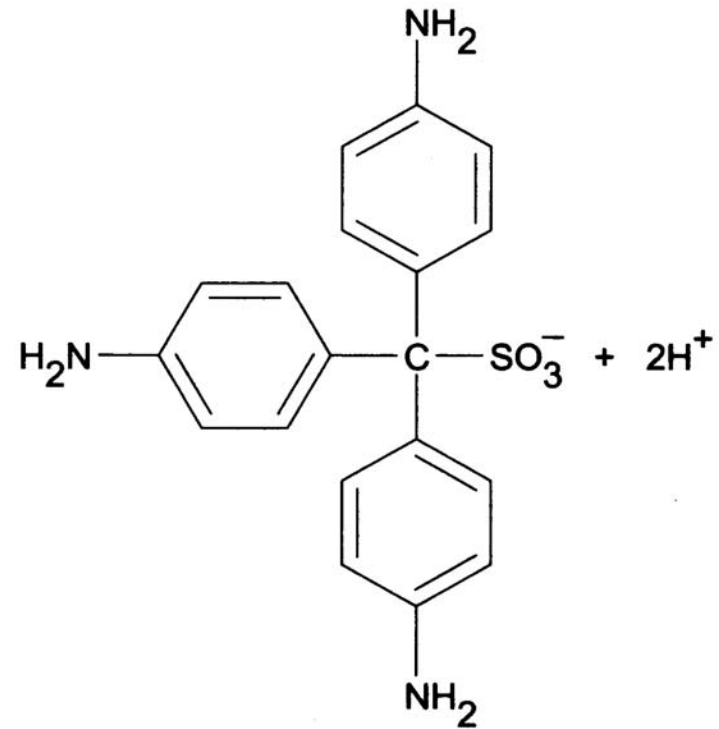


# mor Some Chemistry...

acid fuchsine



Schiff's reagent



# Some Aspects of Dye Batches

- 3 different types of basic fuchsine powders
  - either
    - nearly all (only) pararosaniline
    - mainly new fuchsine
    - any combination/permutation of 4 homologues
- Pure (*single*) dye vs. chemically pure
  - salts, dextrans & unknowns
  - Biological Stain Commission
    - certifies pararosaniline (single) or basic fuchsine @ 88%
    - $\text{TiCl}_3$  assay

# Applications for Basic Fuch sine

- Nuclear and mucin staining
- Acid-fast / carbol fuch sine methods (e.g. Ziehl-Neelsen).
- Preparation of Schiff's reagent (periodic acid Schiff (PAS)/Feulgen)
- Preparation of aldehyde fuch sine
- Preparation of resorcin fuch sine
- Preparation of hexasonium salt
  - enzyme histochemistry and immunolabelling
- Microbiological media
  - Endo medium
- Flagellar staining (pararosaniline, 3:1 acetate:chloride)

**CAUTION**  
Some methods stipulate  
specific homologues

# Why Advice can be Misleading

- Very few studies have used chemically pure homologues of basic fuchsin

- Schulte E, Wittekind D. (1989) Standardization of the Feulgen-Schiff technique. Staining characteristics of pure fuchsin dyes; a cytophotometric investigation. Histochemistry 91: 321-331

Property	Pararosaniline	Rosaniline	Magenta II	New Fuchsin
CI number	42500	42510	No number	42520
CI name	Basic red 9	Basic violet 14	Not cited	Basic violet 2
M.W. chloride salt	323.73	337.86	351.87	365.91
$\lambda$ max (95% ethanol) nm	542	544	551	554
Assay $\epsilon$ $\times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$	8.57	8.53	8.62	8.68

# Why Advice can be Misleading

- Purity often determined utilising spectrophotometric methods
  - limitations for mixtures
    - need to know if single dye by chromatography
- Staining recommendations come from studies which do not use pure dyes
  - Don't know
    - which homologues
    - composition of non-dye components
    - concentration

# Do we want pure dyes?

- Standardise methods / procedures
  - economic costs prohibitive
  - approximate standardised reagents
    - certified dyes (external or internal)
    - reasonably reproducible staining
      - quality assurance on stained result
      - outsourced staining solution production
- Understanding on how/why stains work , or don't...
  - e.g. acid-fast staining

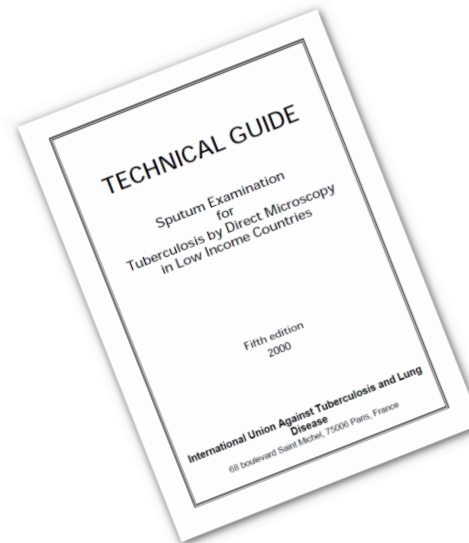


# WHO

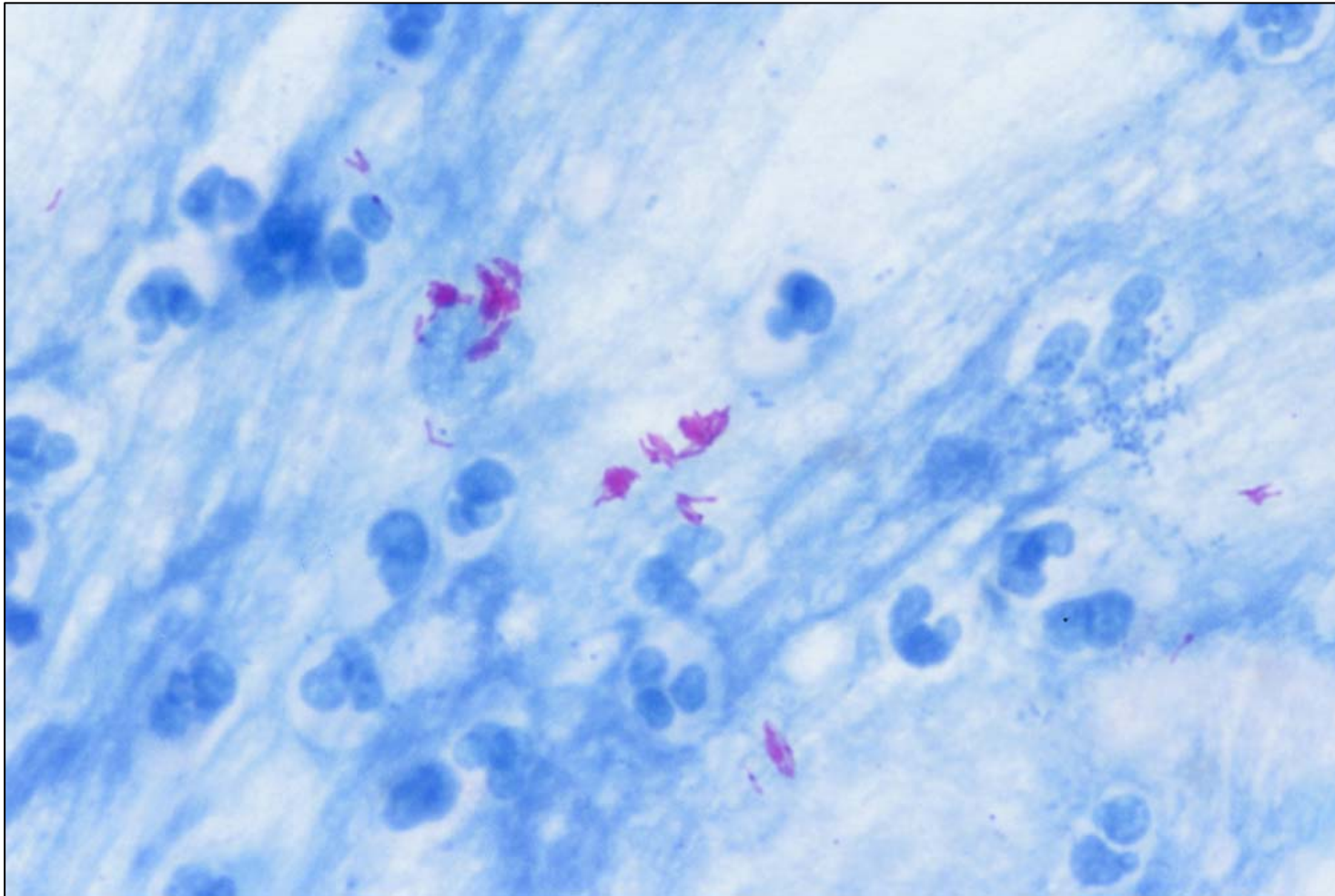
- Tuberculosis programmes
  - Directly Observed Treatment, Short Course (DOTS)
    - containment / treatment for tuberculosis
    - cost effective programmes in resource challenged countries
  - Diagnose sputum smear positive by acid-fast staining
    - sensitivity of test vs. robustness
    - minimal expertise (training) / facilities
    - performance of testing in field under sub-optimal conditions
    - source reagents locally, especially powders

# Acid-fast Staining of Smears

- Ziehl-Neelsen
  - robust
    - consistently good results in field conditions
  - no special equipment involved (...microscope)
  - reagents often prepared in “National Reference Laboratory”
  - guidelines



# Biological Staining

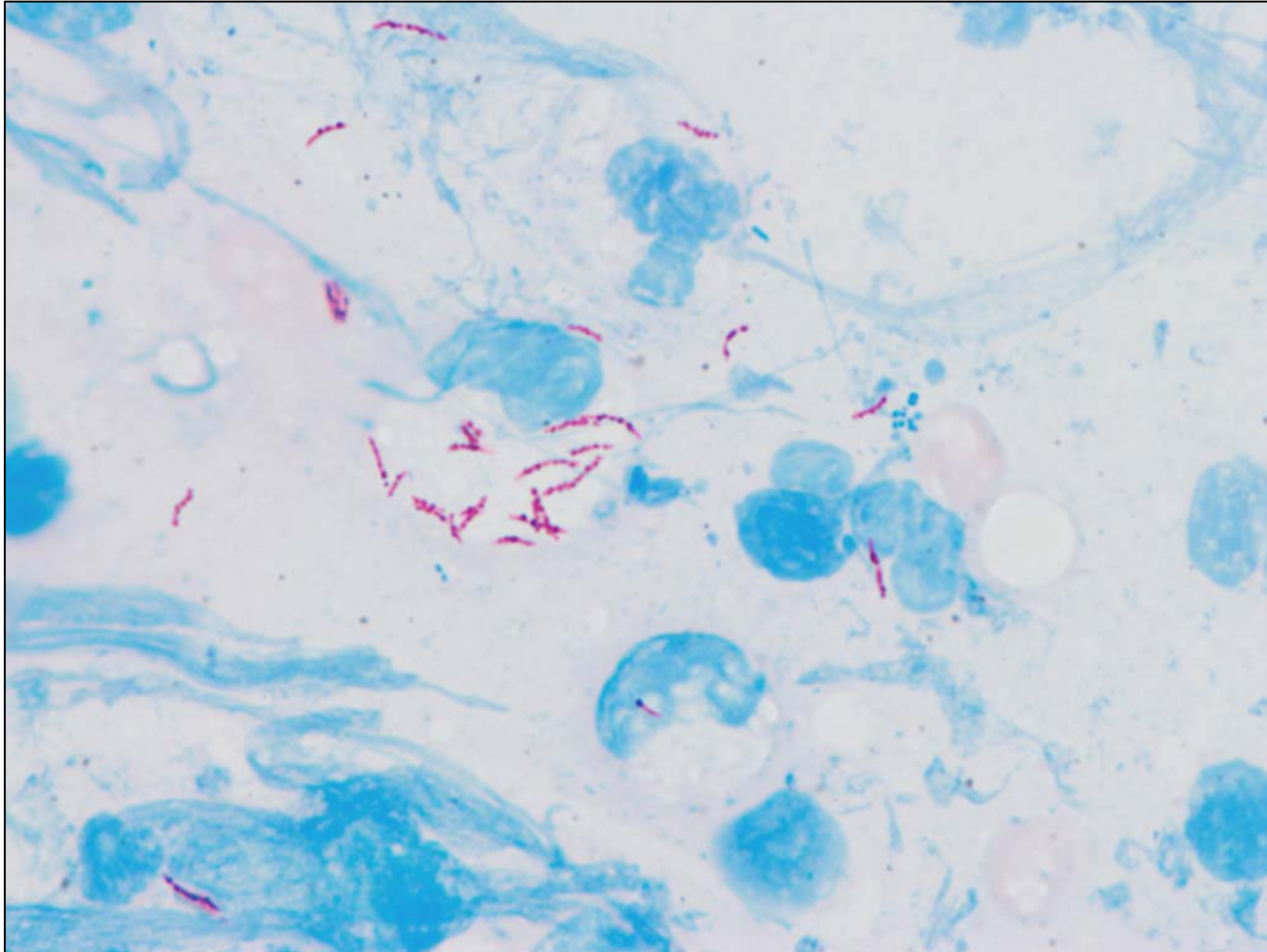


Sputum smear: Ziehl-Neelsen stain *courtesy R. Lumb, Mycobacterium Reference Laboratory, SA Pathology, Adelaide*

# Failure of Laboratory Testing

- Often attributed to selection of non-certified, unspecified, dye powders
  - failure to stain AFB's
  - dirty background / precipitation
  - beading artifact
  - rapid fading of stained smears
- Source of powders not likely to change

# Beading Artifact



Sputum smear: Ziehl-Neelsen stain *courtesy R. Lumb, Mycobacterium Reference Laboratory, SA Pathology, Adelaide*

# Investigation

- Why did this batch fail to stain?
  - is it basic fuchsin?
    - chromatography and spectroscopy
  - does it stain smears in an Australian reference laboratory
    - stain positive control material
  - what is the concentration of dye?
    - how can we measure it? does it matter?
  - what else is present in powder
    - impact on staining

LABORATORY REAGENT  
**RANKEM**  
 FUCHSIN BASIC  
 $C_{20}H_{20}ClN_3$   
 M.W. 337.84  
 Product Code: F0150  
 Net: 25 gm  
 GOSSMOS

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AVONCHEM  
 FUCHSIN BASIC  
 C.I. 42510  
 BATCH NO: 3932-2637  
 EXPIRY DATE: 15/10/27  
 SHIPPING NAME  
 TESTED FOR LABORATORY USE ONLY

25 GMS.  
**LOBA CHEMIE**  
 Art. 3886  
 Fuchsin Basic  
 for Microscopy  
 $C_{20}H_{20}ClN_3$   
 C.I. No. 42510

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**WINTHROP**  
 BRIXWORTH · NORTHANTS · UK  
 Basic fuchsin  
 for microscopical staining  
 CI 42510  
 Produced in the U.K.

碱性品红  
 Fuchsin basic  
**CHEMICAL REAGENT**  
 $C_{20}H_{20}ClN_3$  和  $C_{19}H_{19}ClN_3$  分子重: 337.84  
 灼烧残渣不大于 14.0%  
 合格  
 天津精细化学有限公司

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**DUMKOR**  
 FUCHSIN (BASIC)  
 Prod. No. 39402 25 g  
**SELKROM**

LABORATORY REAGENT  
 NOT FOR MEDICINAL USE  
 25 gm  
**Basic Fuchsin**  
 C.I.No.42510

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CODE 1510370  
 FUSCHINE  
 MAGENTA  
 Lot. 980149 EXP 02/2001  
**LUDECO**

25g 碱性品红  
 Fuchsin basic  
**CHEMICAL REAGENT**  
 $C_{20}H_{20}ClN_3$  分子重: 337.84  
 $C_{19}H_{19}ClN_3$  分子重: 323.83  
 佳惠

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coopération pharmaceutique française  
 77000 melun  
 Contrôle: 04 / 93 09010  
 93074 - 58  
 Fuchsine  
 bactériologique  
 Lot n° F 16418 100g

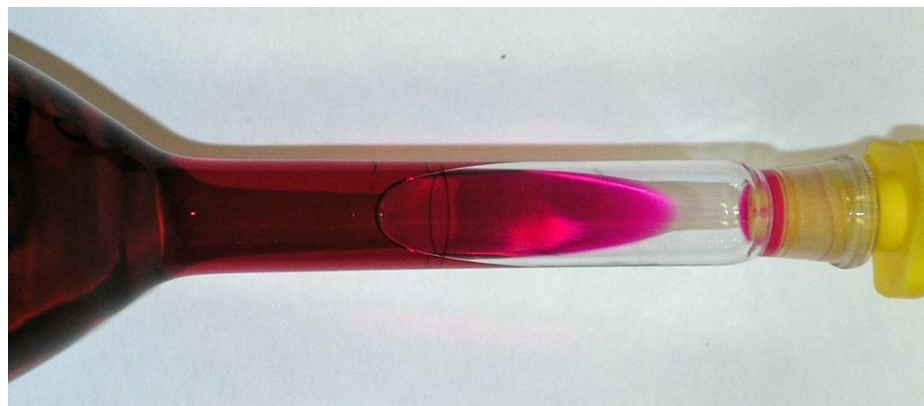
**HIMEDIA** RM 112-10G  
**Basic fuchsin, Certified**  
 Rosaniline  
 C.I. No. 42510  
 $C_{20}H_{20}ClN_3$  Mol. Wt. 337.86  
 CAS No. 532-99-5  
 09014

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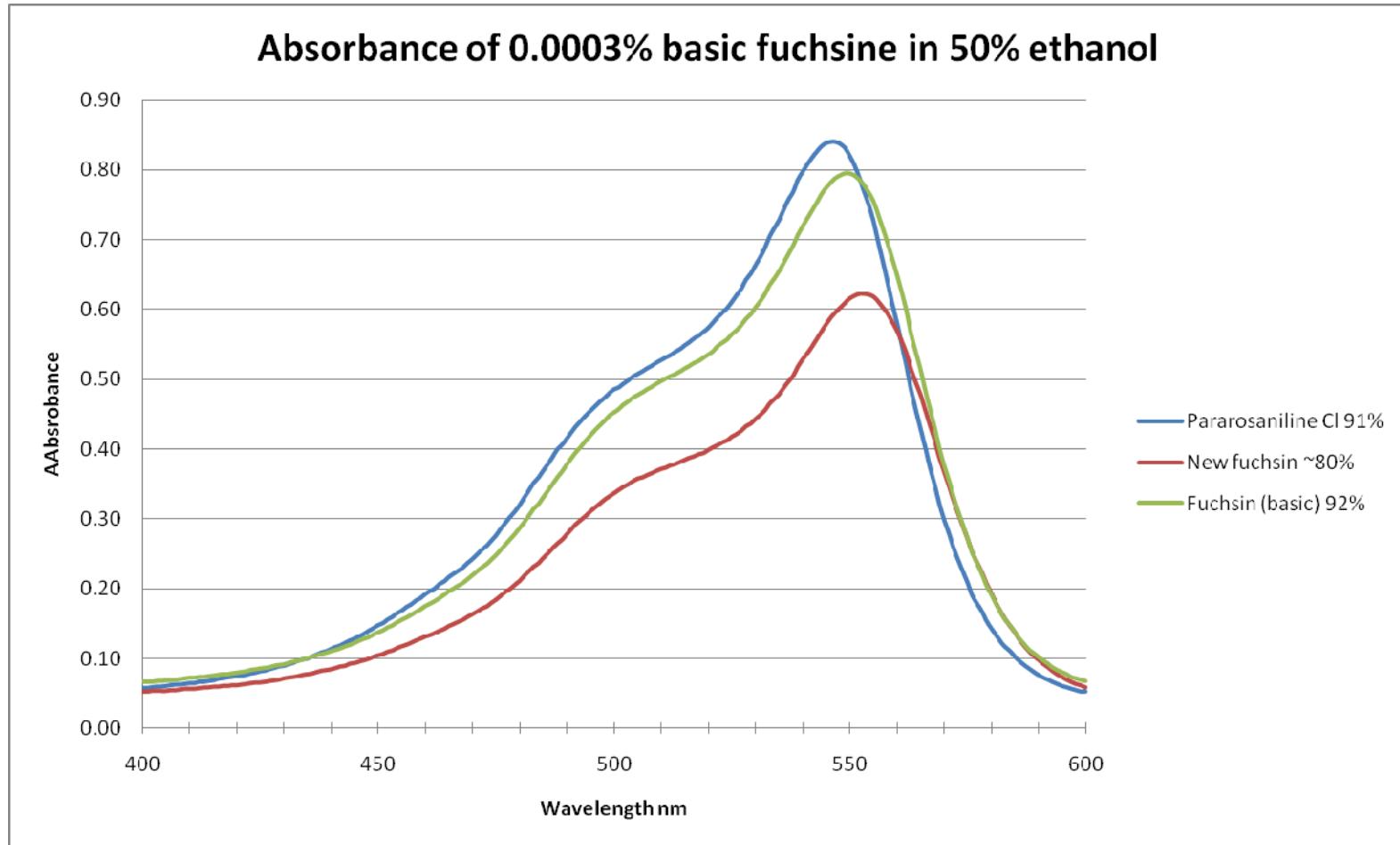
**CDH** Laboratory Reagents  
 Fuchsin (basic)  
 For microscopical staining  
 C.I. 42510  
 25 GM 04039  
 Batch No. Product No. 004039

# Is it Basic Fuchsine?

- Spectrophotometry
  - characteristic signature for basic fuchsine
    - (UV and visible)
  - determine any coloured impurity
  - doesn't resolve which homologues are present
  - a rough guide as to purity



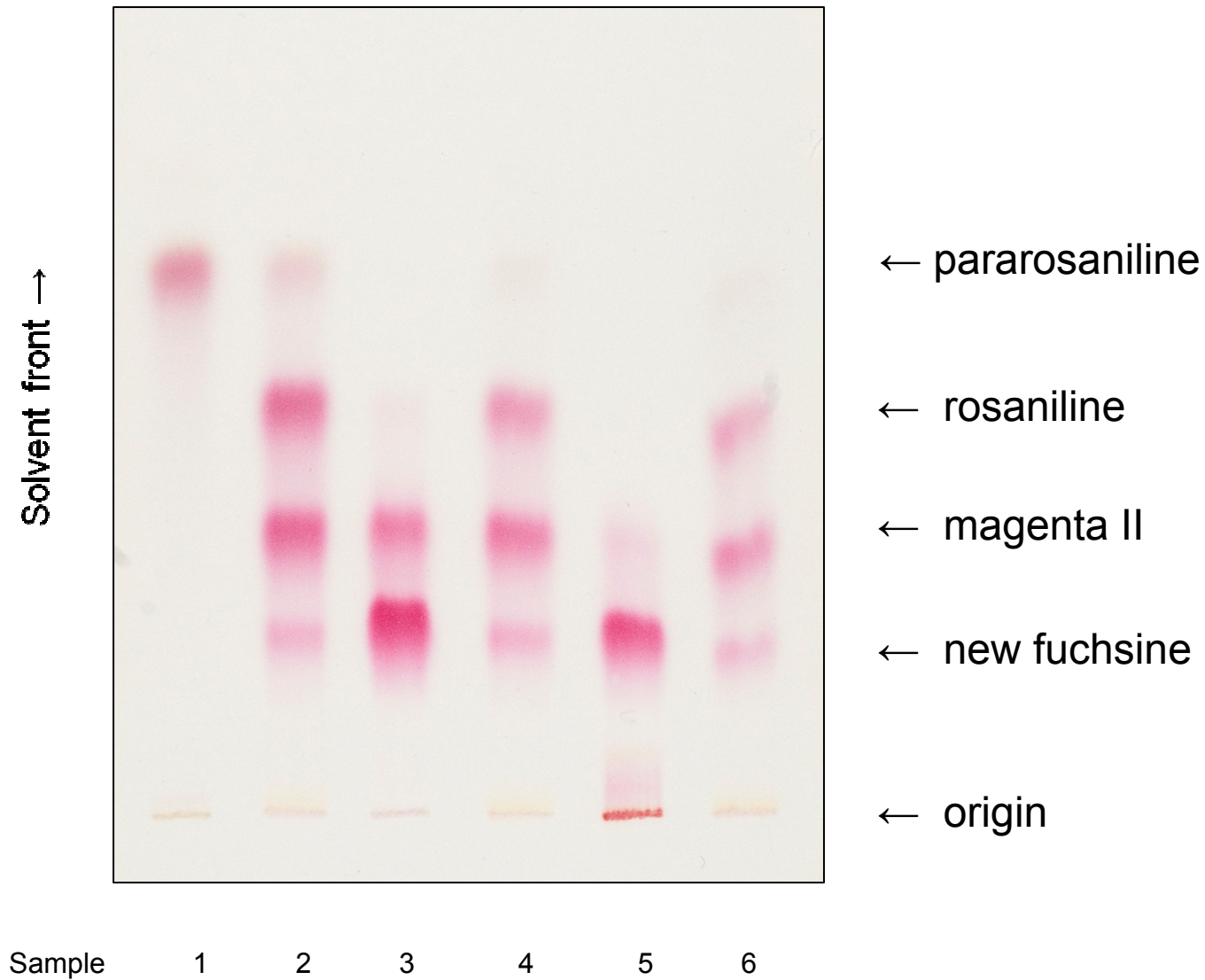
# Spectrophotometry



# Is it Basic Fuchisine?

- Chromatography
  - determine which of the homologues are present
- Quantitative
  - High-Performance Liquid chromatography (HPLC)
    - mass spectrometry
- Qualitative
  - Reverse Phase Thin layer Chromatography (RP-TLC)

# RP-TLC



# Biological Staining for AFB's

- Best indicator for success as stain
  - effect of non-dye elements on staining
  - indication of effective conc. of dye in solution
- Can we determine robust batches of powders?
  - change staining conditions
    - different concentration of basic fuchsin / salt
      - 1%, 0.3%, 0.1%
    - omit/modify various steps
      - phenol, heat, solvents, incubation time

# Outcomes from Investigation

- What criteria can you give for selecting an appropriate dye powder (and its use)?
  - testing within realms of capacity of national programs

# Collaboration

- Richard Lumb
  - SA Pathology (RAH), Adelaide, South Australia
- Armand Van Deun
  - International Union Against Tuberculosis and Lung Disease, Paris, France