

# TRACKING A MALO-LACTIC FERMENTATION

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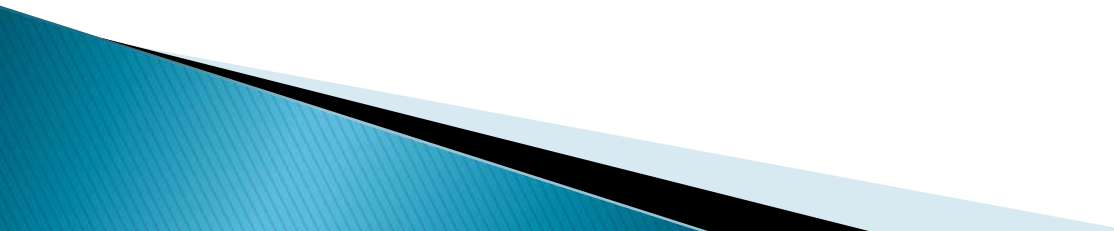
**VESTA – VIN 268 Instructor**



# Monitoring MLF

- ▶ MLF – easily monitored by paper or thin-layer chromatographic separation
  - absence of a malic acid spot
- ▶ Visual resolution for malic acid limits at approximately 100 mg/L
  - most winemakers prefer 15 to 30 mg/L malic acid to consider their wine “safe”

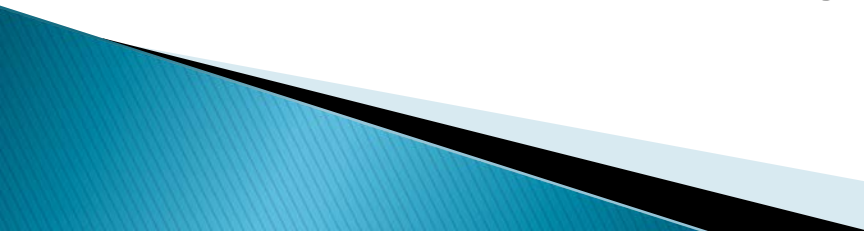
# PAPER CHROMATOGRAPHY

- ▶ Whatman No.1 chromatography paper
  - ▶ Chromatography developing tank
  - ▶ Separatory funnel
  - ▶ Micropipettes (20 mL)
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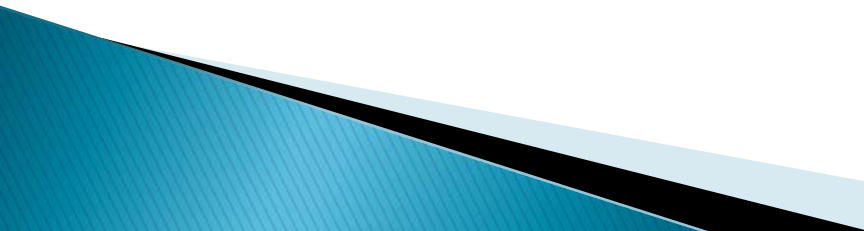
# PAPER CHROMATOGRAPHY

- ▶ Wine acid standards (0.3%) purchased as part of kit or made up
- ▶ Chromatography solvent – can be purchased commercially
  - ▶ 100 mL n-butanol
  - ▶ 100 mL de-ionized water
  - ▶ 10.7 mL stock formic acid
  - ▶ 15 mL indicator solution prepared by dissolving 1 g of water-soluble bromocresol green in 100 mL of de-ionized water.
- ▶ Shake solvent mixture thoroughly by repeated inversion of separatory funnel. Allow for phase separation, and discard lower phase.
- ▶ NOTE!! Solvent should be prepared fresh on a weekly basis.

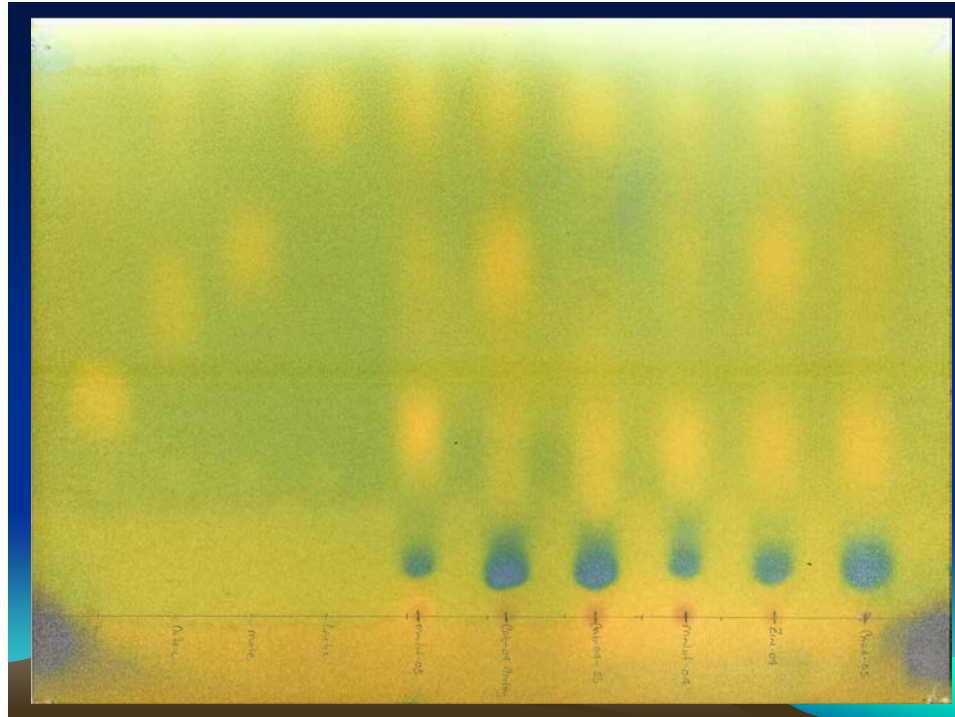
# PAPER CHROMATOGRAPHY

- ▶ Taking care to handle chromatography paper only by the edges, cut a piece of appropriate size to fit into developing tank.
  - ▶ Using a pencil, draw a line parallel to, and approximately 2.5 cm from the bottom edge of the paper.
  - ▶ Using micropipettes, spot standard acids and wine samples at equal intervals along baseline. Spots should be of as small a diameter as possible (less than 1 cm). Re-spot at least twice in order to achieve this goal.
  - ▶ Each spot should be at least 2.5–3.0 cm apart. A hair dryer can be used to assist in drying the spots between applications.
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# PAPER CHROMATOGRAPHY

- ▶ Transfer solvent to developing tank, allowing at least 30 min for vapor saturation to occur (shake tank).
  - ▶ A minimum depth of 0.75 cm of solvent is required for adequate development.
  - ▶ Immerse baseline side of paper into tank, taking care that solvent moves uniformly up the paper.
  
  - ▶ When the solvent has ascended to near the upper edge of paper, chromatogram may be removed and allowed to dry.
  
  - ▶ When dry, results may be interpreted by noting the positions of yellow spots (acids) on blue background. Identification of various wine acids may be made by comparison to standard acids
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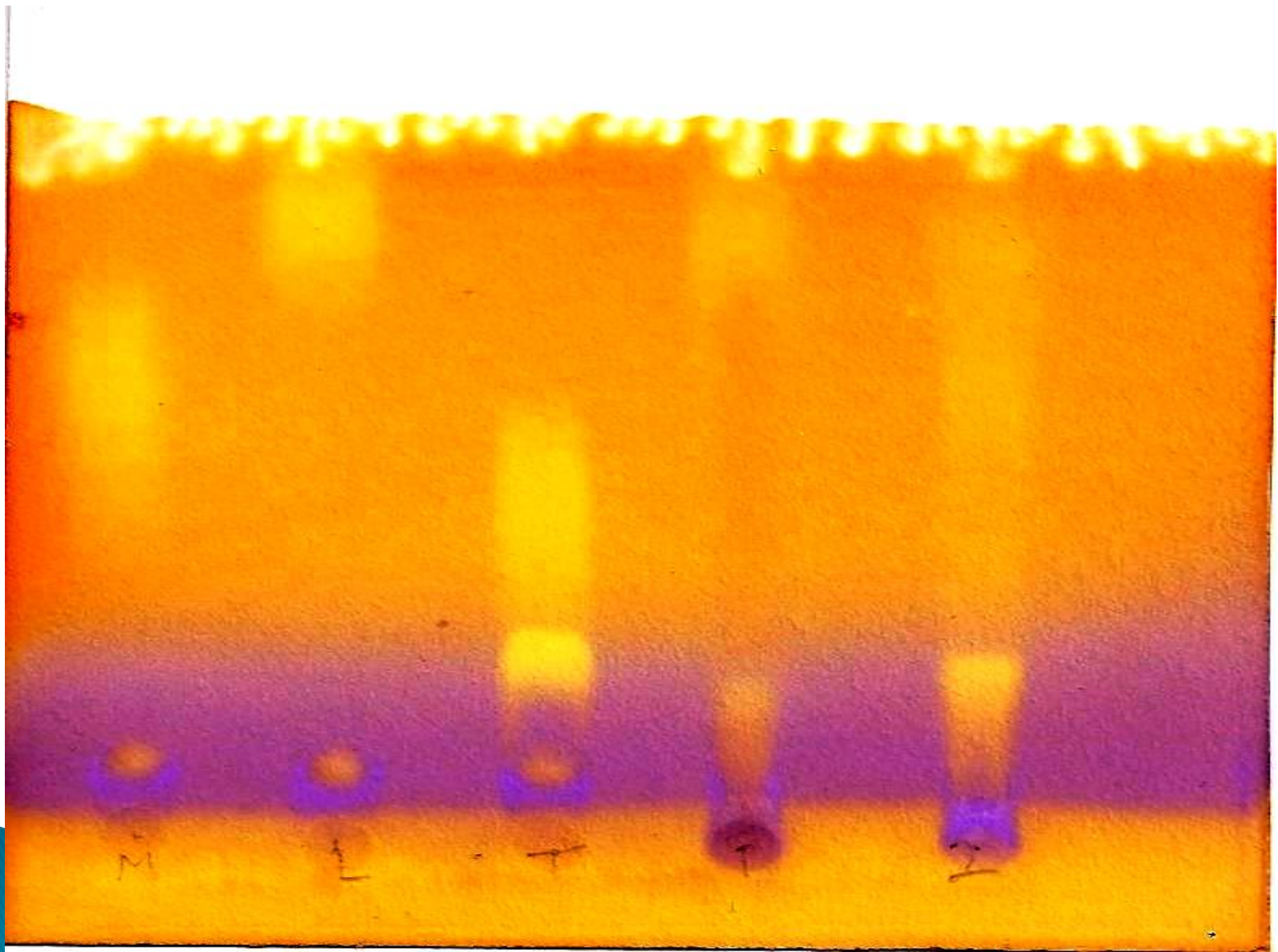
# Results



# TLC – MALIC ACID

- ▶ I. J. T. Baker pre-coated plastic chromatography sheets with microcrystalline cellulose (J. T. Baker 4480 20x20 cm or equivalent)
- ▶ Chromatography developing tank
- ▶ II. Wine acid standards (0.5%): malic, lactic, and tartaric acids
- ▶ III. Chromatography solvent: In a separatory funnel, mix the following:
  - ▶ 100 mL n-butanol                      100 mL deionized water
  - ▶ 10 mL stock formic acid                      0.1 g bromphenol blue indicator
- ▶ IV. Spot 10  $\mu$ L standard acids and wine samples at equal intervals along baseline. Spots should be of as small a diameter as possible. Let spots dry and re-spot another 10  $\mu$ L. A hair dryer can be used to assist in drying the spots between applications





# Enzymatic Malic Acid

- ▶ Limitations on paper chromatographic method ~ 100 mg/L
- ▶ Available kits
  - Production of NADH
  - Small sample volumes
  - Need to run standards