

RHINEHART DEVELOPMENT CORP  
DAIRY RESEARCH PRODUCTS, INC.  
5345 COUNTY ROAD 68  
PHONE: 260-238-4442  
FAX: 260-238-4402

**Estimation of somatic cells in milk by means of the  
Wisconsin Mastitis Test Equipment**

1. Test tubes\*, clear plastic, flexible, measuring 12.1 x 130 mm I.D. having a 3 mm (approx.) Diameter air vent about 65 mm from the outside bottom of the tube. Do not store tubes with caps on. If caps become loose preferably replace tubes or place a layer of scotch magic mending tape around top of tubes. Insert tubes from bottom of rack. Calibration test: WMT tubes have correct inside dimensions when the combined height of the column of 2ml of milk plus 2 ml of reagent is 37-38mm. Use glass pipettes of known accuracy. Discard tubes when they fail to meet test.
2. Caps\*, metal with 1.09 - 1.10 mm diameter orifice in center made of 25 gauge (0.5 mm) brass.
3. Racks\*, to hold firmly 12.1 x 130 mm plastic tubes.
4. Automatic 2 ml syringe\*\* for pipetting milk samples. This is assembled from b-d syringe no. 1250s, b-d metal holder no. 1250 ML and laboratory cannula 2 to 4 inch long. Syringe is properly adjusted when 2 ml volume is .20 mm high in calibrated WMT tube.
5. Cornwall continuous pipetting outfit 2 ml size\*\* b-d no. 1251 for pipetting reagent. Syringes should be adjusted and the accuracy checked by collecting delivered volumes in a graduate cylinder. Or, correct adjustment is indicated when column of 2 ml milk plus 2 ml reagent is 37 mm high in calibrated WMT tube.
6. Two laboratory cannulas\*\* b-d no. 1250 NR, 14 gauge 4 inch long. These may be cut to desired length with a hacksaw or file. One cannula is connected to the 2 ml syringe and the other, approx. 3-1/4 inch long, is connected to the Cornwall continuous pipetting outfit described above. This latter cannula should extend at least 1/4 inch below the level of the surface of the milk sample when the pipetting outfit is resting on the two-way valve.
7. Clock with circular dial and sweep second hand extending over marks on dial.
8. Measuring square\* calibrated to read directly in somatic cells per ml. Or millimeter square\* for reading in WMT valves.

#### Reagent

Preferably use standardized WMT reagent\* for greatest accuracy and uniformity (dilute concentrate in small quantities as needed according to proportion indicated on bottle.) Caution: old solutions of ready-to-use reagent may contain high bacterial populations that may affect test.

#### Procedure

1. Rinse tubes and shake to remove excess water before using (or before calibrating). Pipette 2 ml of well-mixed milk samples into each respective tube in one or more racks. Syringe is rinsed in water and in the milk sample before pipetting from it. Samples should be kept at 32 - 40 f and tested preferably on day of collection. If necessary to hold samples overnight. They should be packed in ice to assure adequate refrigeration. WMT reaction decreases slowly in storage. Avoid freezing of samples; (note) samples should be tested promptly after removal from refrigeration the first time. Samples that have warmed above 40 f. Replaced in the refrigerator and used later for the WMT will not give reliable results.
2. Quickly add two 1-ml portions of reagent to each of the tubes in one rack. Reagent is delivered below the surface of milk with cannula attached to Cornwall continuous pipetting outfit. Reagent is added in two 1 ml volumes to provide good initial mixing of milk and reagent. The cannula is inserted into the test tube until the pipetting outfit rests on the arm of the two-way valve. • Cannula should be long enough to reach at least 1/4 inch below the surface of the milk sample. This eliminates foaming, (the carry-over on the cannula between tubes appears to be insignificant. Caution: mechanical pipetting is essential to provide rapid, forceful addition of reagent.

3. Promptly cap tubes with caps previously arranged right side up in front of rack.
4. For greatest precision the mixing action should be started about 30 seconds after adding reagent to first tube hi rack. Mix by holding tubes nearly horizontally and tilting rack back and forth permitting the liquid to run forward to the caps 10 times and return. The ten excursions should be made in 8 - 10 seconds. During the forward tilt of the tubes the more fluid samples in the rack are allowed to cover about  $1/4$  of the cap. During the backward tilt the butts of the tubes move down in an arc of about  $1/4$  inch from the horizontal. Avoid vigorous agitation. The following bit of information should be helpful in establishing correct technique: to guide tilting movement, place back of WMT rack on top of a 5-6 mm diameter rod or a 1ml milk pipette lying on the laboratory table. The rod acts as a \*\*\*rocker and should reduce the tendency to tilt tubes backwards excessively. Avoid touching the tabletop with the rack during forward and backward tilt.
5. Make any needed adjustments such as straightening caps on the tubes after the mixing action. At this stage the viscosity is relatively stable.
6. The rack is inverted within 30 seconds after mixing action. Prior to inversion the tubes are held hi a horizontal position while waiting for the sweep second hand on the timer to reach a convenient starting point. The rack is then inverted rapidly but smoothly and held in a vertical position throughout a 15-second outflow. The tubes are returned to an upright position and allowed to stand for at least 1 minute to allow liquid to drain down before reading. The temperature of the mild-reagent mixture at the time of inverting is  $24 \pm 2$  c (72 - 79 f). Temperature control may be accomplished by warming reagent to about 35 c (95 f) by placing hi water in large styrofoam picnic jug when testing samples directly from refrigerator. The caps are removed immediately after use and cleaned by placing in a small container of warm water and shaking them in two changes of water.
7. Results may be read directly hi somatic cells per ml by using direct reading measuring square. The results are reported hi WMT somatic cells per ml or hi WMT valves (if the mm-measuring square is used).
8. The tubes are rinsed 2 or 3 times hi warm tap water not over 45c-(113 f), after each use. Excess water is removed by shaking rack before each use.

Calibration of caps - preferable use special WMT nozzle gauge\* - correct size of orifice is indicated is when end of WMT gauge wire may be inserted into orifice about 1.5 to 2.5 mm without pressure.

\*\*\*Latest WMT racks have a slight bend placed hi the back of the rack that serves as a rocker.

! Original method was published under the title "the Wisconsin mastitis test - an indirect estimation of leukocytes hi milk" D. I. Thompson and D.S. Postle, Journal of Milk & Food Tech., Sept. 1964, vol. 27. No 9 pg 271 - 275 (revised by D.I. Thompson, May 1971.)