MLAB 2360 Clinical I
Activity 7: Immunodiffusion Tests

Objectives:

1. Describe the principle(s) of immunodiffusion testing.
2. Determine the concentration of an immunoprotein by preparation of a reference curve and determining the patient, control or unknown's values from the curve.
3. Recognize the reactions of identity, non-identity and partial identity.
4. Evaluate instructional materials to determine the substance being analyzed, the principle of the procedure, the expected value, significance of abnormal results, limitations of the procedure, and troubleshooting procedures to follow when control results are unacceptable.
5. Appropriately record and report results as instructed.
6. Utilize lecture notes, textbook and laboratory information to answer study questions.

Introduction:

Gel precipitation is an immunologic assay in which soluble antigen and antibody are allowed to diffuse through a gel medium. As the antigen and / or antibody diffuse from the point of application (usually a well cut in the agar) through the gel, their concentration decreases until they eventually arrive at their own zone of equivalence (optimal concentration of antigen and antibody) and forming visual precipitation within the gel.

Explanation of terms often applied to gel diffusion tests:
Diffusion - refers to how many reactants are moving through the gel. Single-diffusion tests have only one (1) reactant (usually the antigen) moving, while the other reactant is fixed in the gel. Double-diffusion testing has both of the reactants moving though the medium.
Dimension - refers to the direction of movement. Reactions in tubes essentially have only one effective dimension, up and down and are called single dimension. When a reactant is able to move both up and down as well as radially through a gel the term double dimension can be applied. Therefore using this criteria, there are four combinations or classification of reactions in gels:

1. single diffusion - single dimension
2. single diffusion - double dimension
3. double diffusion - single dimension
4. double diffusion - double dimension

Principle:

When antigens and corresponding antibodies are allowed to react in gels or other mediums, they will diffuse toward one another, and at the point in which they meet in optimal proportions, they will form a visible precipitate.

Ouchterlony gel diffusion (double diffusion, double dimension)
When an antigen solution is placed in a well / hole cut into an agar plate and the corresponding antibody is placed in an adjoining well, they will diffuse radially from their respective wells toward one another. When an optimal ratio of the two materials is reached, a visible line of precipitation is formed in the gel. The test provided *qualitative* identification of individual proteins.

Radial immunodiffusion (RID) (single diffusion, double dimension)
RID testing is used to obtain a *quantitative* level of an antigen when agar containing an appropriate antiserum (antibody is "fixed" in the agar) is poured in plates and carefully cut circular wells are removed. A series of three (3) standards containing known concentration of antigen are placed in three of the wells, while control and "unknown" samples are placed in other wells. As the antigen diffuses radially, a ring of precipitate will form in the area of optimal antigen - antibody concentration. A standard curve is prepared using the ring diameters of the standards versus their concentrations. This curve is then used to determine the concentration of the control and unknown samples.

RADIAL IMMUNODIFFUSION
(dry lab determination of IgG concentration)

Summary and Explanation:

The relationship of immunoprecipitation ring size and antigen concentration was described in the mid-1960s by Mancini et al. Mancini observed that the precipitin ring diameter stopped increasing at the point where diffusible antigen had been reduced and antigen-antibody complexing had attained equivalence. At equivalence, or endpoint, a linear relationship exists between the antigen concentrations and their corresponding ring diameters squared. A reference curve on linear graph paper is constructed by plotting the square of the precipitin ring diameters of reference sera against their corresponding concentrations.

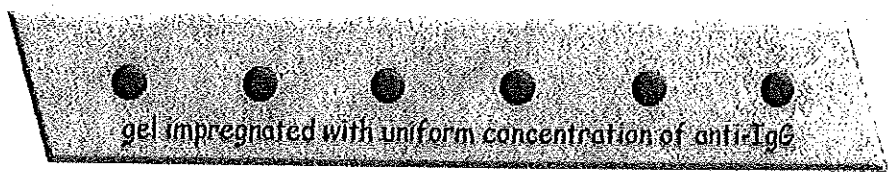
Materials:

1. Lab paper representing the RID rings of standards, controls, and patients.
2. Ruler capable of providing accurate measurement in millimeters.
3 Calculator
4 Linear graph paper

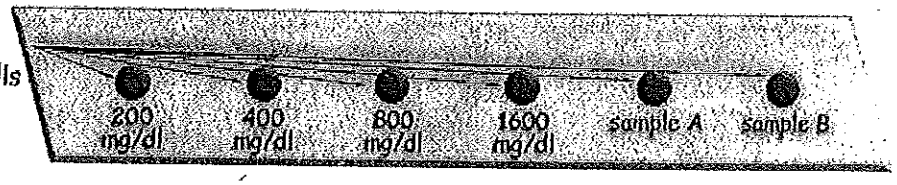
Procedure:

1. For each diagram of a precipitin ring below, measure the diameter in millimeters and record the results in the space provided.
2. Square the diameter of the precipitin ring and record in the space provided.
3. Use the graphing paper provided, create a standard curve of the results of the standards provided.
4. Read the results of the controls and patients from the standard curve.
5. Record the results in the spaces provided using correct units.
6. Using product insert or other reference information, determine acceptability of the controls. Indicate your evaluation of each control by circling YES or NO.
7. Using textbook or other references, evaluate each patient result as to being low, normal, or high. Indicate your evaluation of each patient by circling the appropriate response.

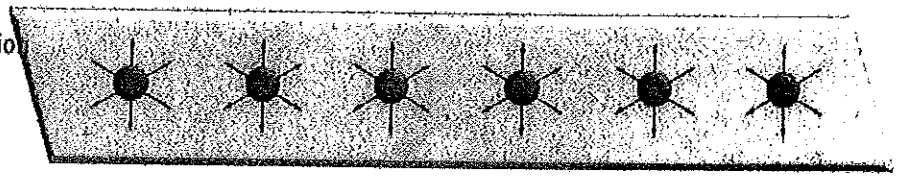
REAGENTS:
agar gel impregnated with antiglobulins (anti-IgG)



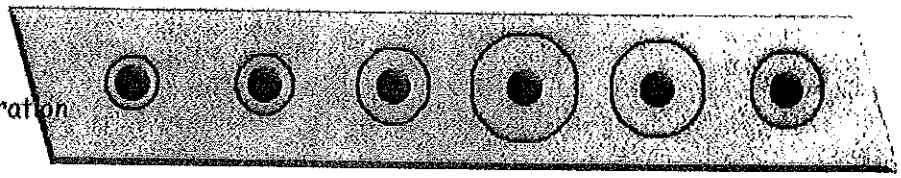
1. add serum sample & IgG standards to wells



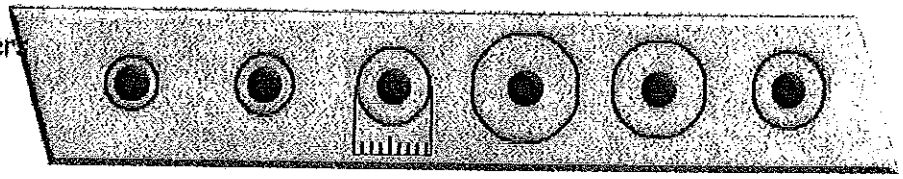
2. allow time for diffusion of IgG's into gel

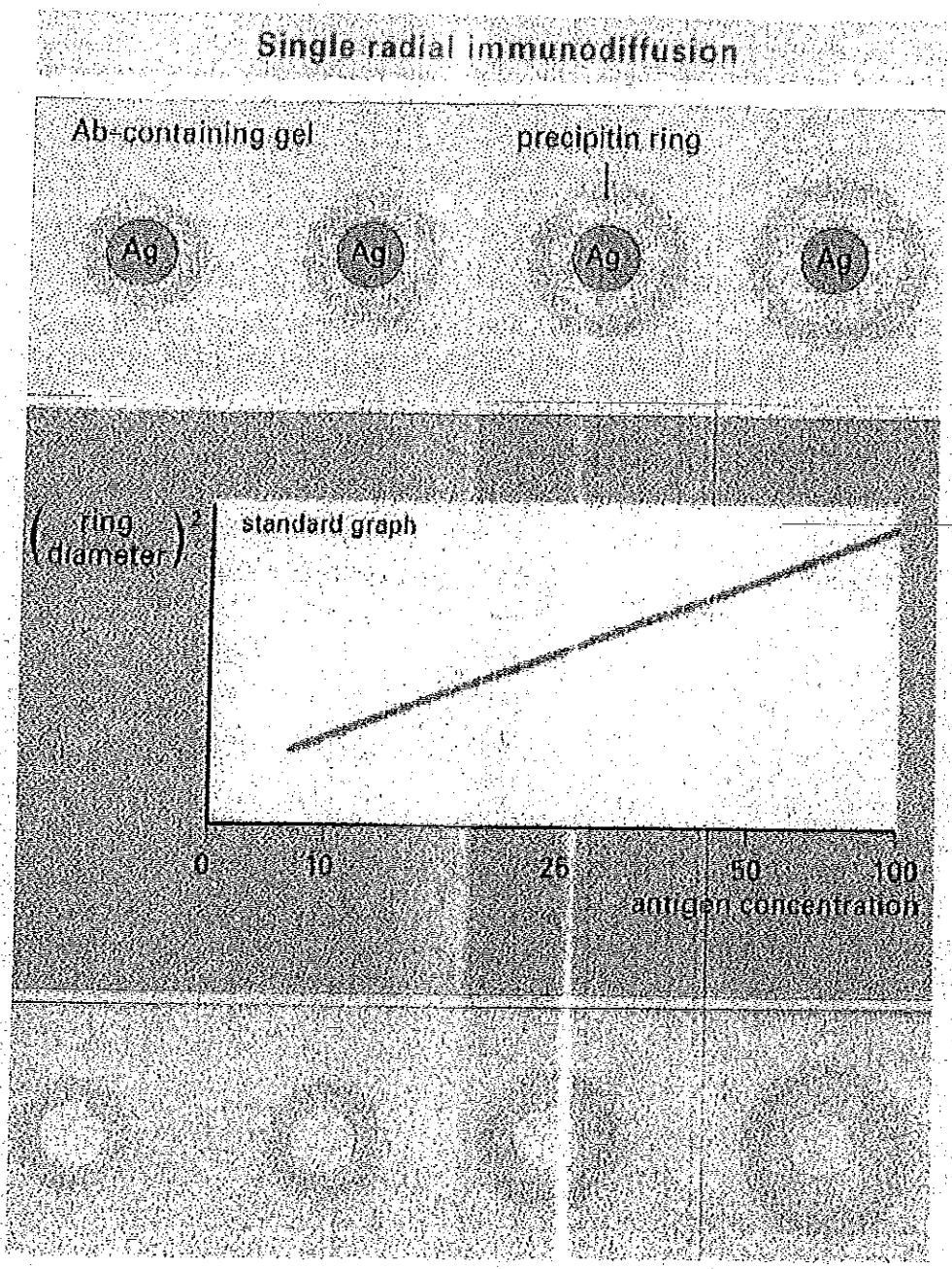


3. precipitin rings form at site of optimal IgG:anti-IgG concentration



4. measure ring diameter (proportional to IgG concentrations)



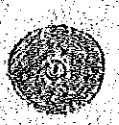
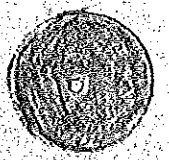

Single radial immunodiffusion
Ab-containing gel
precipitin ring
Ag
Ag
Ag
Ag
(ring diameter)²
standard graph
0
10
26
50
100
antigen concentration

Name _____

RADIAL IMMUNODIFFUSION
DRY LAB

1. Measure each of the precipitin rings and record the results in the space provided.
2. Square the diameter of the precipitin ring and record in the space provided.
3. Create a standard curve using the results of the standards provided.
4. Read the results of the patients and controls off the standard curve.
5. Record the results in the spaces provided.

Standards:

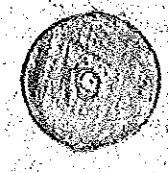




Ring Size _____ Ring Size _____ Ring Size _____
Size Squared _____ Size Squared _____ Size Squared _____
Value 50 mg/dL Value 100 mg/dL Value 200 mg/dL

Controls:

LOW NORMAL HIGH





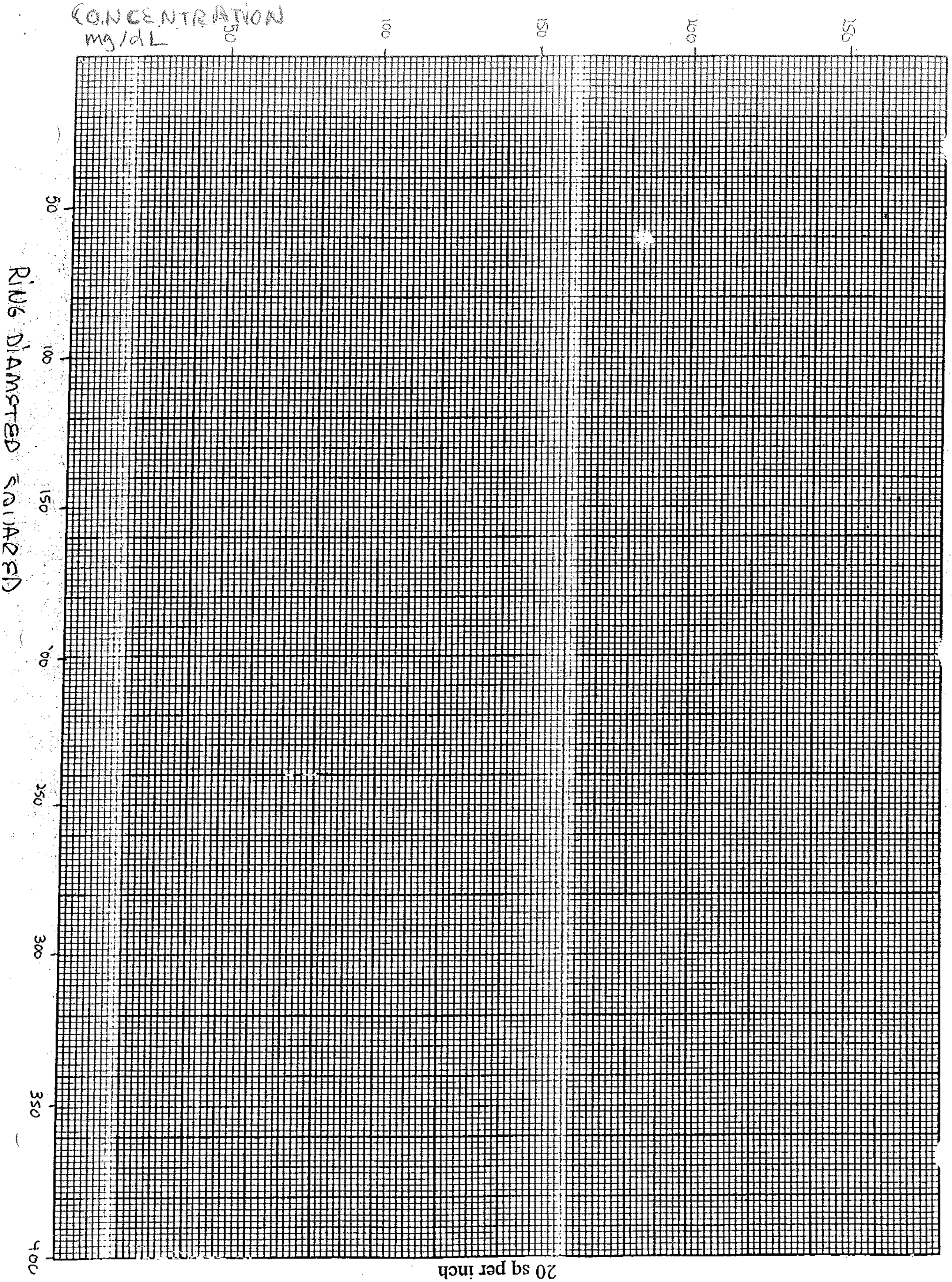
Ring Size _____ Ring Size _____ Ring Size _____
Size Squared _____ Size Squared _____ Size Squared _____
Range: 40-79 /dL Range: 80-195 /dL Range: 196-250 /dL

Patients:

#1 #2 #3



Ring Size _____ Ring Size _____ Ring Size _____
Size Squared _____ Size Squared _____ Size Squared _____


CONCENTRATION mg/dL
50
100
150
200
250
RING DIAMETER SQUARED
50
100
150
200
250
300
350
400
20 sq per inch

MLAB 2360 Clinical I
Activity 7: RID - dry lab
Recording/Interpreting Results

Name _____ Date _____

Instructions:

1. Read the values for the controls and patients off of the standard curve that you created and record them below **USING APPROPRIATE UNITS**.
2. Indicate whether the values of the controls are in the acceptable range.
3. Use the values for the normal control.
4. For each patient value indicate whether the values is low, normal or high.

CONTROLS		
	Results	**Acceptable (YES or NO) If "NO" state reason.**
LOW		
NORMAL		
HIGH		

Normal Values = Use values stated for the Normal Control

PATIENT VALUES		
	Results	State whether the results are high, low or normal.
PATIENT 1		
PATIENT 2		
PATIENT3		