Agar Gel Immunodiffusion (AGID) Test
Principles and Techniques

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Agar Gel Immunodiffusion Test

**Definition:**

- The passive diffusion of soluble antigens and/or antibodies toward each other leading to their precipitation in a gel matrix.

**Synonyms:**

- Ouchterlony test (1949)
- Double immunodiffusion test
- Agar gel precipitin (AGP)
Agar Gel Immunodiffusion Test (AGID)

**Advantages:**
- Group-specific test (detects antibodies to all subtypes of flu-A, determine antigenic relationships)
- Easy, requires few reagents/equip

**Disadvantages:**
- Semi quantitative
- Moderate sensitivity
- Subjective interpretation
- Requires 24 hr
100 mm petri dishes – 15-17 ml agar
60 mm petri dishes – 5-6 ml agar
Agar Gel Immunodiffusion Test

Gel Preparation:

- 0.9% agarose in PBS (0.01M, pH 7.2) with 8% sodium chloride

- Dissolve, autoclave 10 min, cool to about 60-80°C and dispense
  - Pour plates on same day used
  - 15 – 17 ml (100 x 15 mm petri dishes)
  - 5 – 6 ml (60 x 15 mm petri dishes)
Template Dimensions

Wells 5.3 mm in diameter

Wells 2.4 mm apart

Agar 2.8 mm thick
Source of Template
Cutters

Veterinary Diagnostic Technology, Inc.
4890 Van Gordon St., Suite 101
Wheat Ridge, Co.  80033
Phone:  (303) 467-2741
Fax:  (303) 467-2799
VACUUM ASSEMBLY

- Single hole stopper
- Side-arm flask
- Glass or plastic tubing
- Vacuum hose
- Vacuum set at 12 to 20 psi
- 12 to 14 gauge cannula
Placement of Reagents/Samples

As = Positive enhancement serum
Ag = Antigen
Numbering Patterns

100 x 15 mm petri dish
Agar Gel Immunodiffusion Test

Reagent Sources (AIV):

• NVSL, Ames, IA:
  ✓ Domestic AIV surveillance – antigen and enhancement serum are available, free of charge
  ✓ Export testing, international – available for sale

• Charles River (SPAFAS), Norwich, CT:
  ✓ AIV antigen and enhancement serum available for sale
Filling Wells

50-60 µl
Cross section of wells showing reagent levels

- Over filled
- Under filled
- Correct
Hold Tip Vertical When Filling Wells
Method Not Recommended
Fill All Wells

PBS

Sample #1

Ag

As

PBS
Diffusion of Reagents

Ag

As

Seen as a precipitin line when concentrations are optimal.
Diffusion of Reagents

At 24 hours a precipitin line is visible
Precipitin Lines After 24 Hours Incubation (Negative samples)
Examples of Positive Reactions

May be seen as light haze

Negative

May be seen as light haze
Examples of Nonspecific Lines

- Negative
- Line of non identity
- Line of partial identity
- Negative
- Negative
- Negative
Incomplete Line Formation

Before 24 hours

At 24 - 48 hours
Potential Positive Sample
Additional Problems
“Halos”
Unbalanced Reagent Concentrations

As too strong

Ag too strong
Cutting Errors

May miss the weak positives