



<b>ATF-LS-E17 Spot Tests</b>	Published Online: <b>March 2018</b>
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**I. Scope:** Used for initial testing/screening of suspected intact and explosives residue. Colors and/or crystalline precipitates resulting from reactions of a known reagent with an unknown solution are used for identification of anions or cations. See attached procedures for each specific spot test.

**II. References:** See attached procedures for references for each specific spot test.

**III. Apparatus/Reagents:**

Spot plates (disposable if possible), glass well slides, disposable pipettes, UV light and hot plate (if needed), gloves and eye protection.

**Standard Solutions and/or Reagents**

Test reagent solutions, known anion or cation standard solutions (see attached procedures).

**IV. Safety Precautions:** Follow chemical hygiene plan. Wear eye protection and gloves.

**V. Procedures:**

**Sampling and Sample Prep**

See the following procedures for the specific spot test to be completed:

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**Analytical Procedure**

Dropwise addition of test reagent to sample extract or solution, in a spot plate or on a well microscope slide. Positive and negative controls must also be prepared. Examine formation of characteristic color or precipitate and record actual observations as well as conclusions in the case notes. See attached SOP for the specific spot test procedure to be completed.

### Non-Routine Exam

If variations in case samples are such that accepted procedures are inappropriate, use discretion in selecting the method most appropriate to the problem at hand.

#### VI. Quality Assurance/Quality Control:

Use disposable spot plates or other supplies whenever possible to avoid contamination. Avoid overfilling the well. Make sure the reagents are not outdated, and that new reagents are properly labeled with date, contents, and examiner initials.

### Ammonium Ion in Water Extracts - Nessler Spot Test

#### A. Principle

Most tests for the ammonium ion are based on detection of  $\text{NH}_3$  liberated in strongly basic media. Ammonia reacts with a mixture of  $\text{KI}$  and  $\text{HgI}_2$  to form an orange-red precipitate of  $\text{HgI}_2 \cdot \text{HgNH}_2\text{I}$ . As a spot test, the reaction directly in alkaline solution produces an orange-brown precipitate indicative of ammonium salts. The reagent may be prepared in the laboratory [2] or purchased pre-mixed. Sulfate and sulfide are reported to interfere but may be avoided by conducting the test in two steps [1].

#### B. Reagents

(a) Nessler's Reagent - A.P.H.A (Fisher SO-N-16 or equivalent)

#### C. Determination

Pipet 1-2 drops of a neutral or slightly acidic water extract solution to a white plastic disposable spot plate. Add 1-2 drops of Nessler Reagent. An orange-brown precipitate is indicative of an ammonium salt.

#### D. Detection Limit:

Reported: 0.3 ppm  $\text{NH}_3$  [1]

#### E. Pertinent References

1. Feigl, F. and Anger, V. Spot Tests in Inorganic Analysis  
Elsevier Publishing Co. New York, N.Y. 1972 pp. 337
2. Benedetti-Pichler, A.A. Identification of Materials  
Academic Press New York, N.Y. 1964 p. 366

### Chloride in Water Extracts - Silver Nitrate Spot Test

#### A. Principle

Silver Nitrate produces turbidity in solution at ppm levels and forms white precipitates with higher levels of two Group 1 anions in acidic medium,  $\text{Cl}^-$  and  $\text{SCN}^-$ . Thiocyanate is readily identified with a ferric chloride test in a separate sample or using acetic acid and peroxide to prevent coprecipitation of  $\text{SCN}^-$  (1).  $\text{SCN}^-$  can also be oxidized to remove. Other Haloanions such as  $\text{Br}^-$  and  $\text{I}^-$  also precipitate

but are colored. Feigl describes use of hydrogen peroxide and 8-hydroxyquinoline (HQ) to eliminate interference by bromide and iodide (1). Sulfide, if present, interferes but can be readily removed.

**B. Reagents**

- (a) Silver Nitrate Solution - 1% aqueous, Dissolve 1 gm ACS Reagent Grade  $\text{AgNO}_3$  in 100 mL of high resistance deionized water. Store in brown bottle.
- (b) Nitric Acid - 6N. dilute 20 mL ACS Reagent Grade  $\text{HNO}_3$  to 50 mL with high resistance deionized water.

**C. Determination**

Pipet 4-5 drops of water extract solution to a  $\frac{1}{2}$  dr vial. Acidify with 1-2 drops of 6 N  $\text{HNO}_3$ . Add dropwise 2-3 drops of 1%  $\text{AgNO}_3$  solution, shake and observe turbidity or precipitate. Very faint turbidity usually lacks analytical significance. If significant precipitate is observed, test for  $\text{SCN}^-$ . If a brown/black precipitate forms, on a second aliquot of the extract, acidify with 4-5 drops of 6N  $\text{HNO}_3$ , heat to boiling for a few seconds, cool and retest with  $\text{AgNO}_3$ . The dark precipitate should not be observed and a white precipitate will be formed if chloride is present.

**D. Detection Limit:** 10 ppm is visible as turbidity.

CRM test 29Aug97 w/ 10ppm standard with  $\text{H}_2\text{O}_2$  and 8-HQ 2ppm (1)

**E. Pertinent References**

- 1. Feigl, F. and Anger, V. *Spot Tests in Inorganic Analysis*  
Elsevier Publishing Co. New York, N.Y. 1972 pp. 638, 178-179
- 2. Benedetti-Pichler, A.A. *Identification of Materials*  
Academic Press New York, N.Y. 1964 p. 389

### Chlorate Presumptive Test - Reduction with Nitrite

**A. Principle**

Chlorates in solution can be reduced to chloride by active reducing agents such as nitrite. The chloride is identified by silver nitrate. A preliminary test is made with  $\text{AgNO}_3$  to observe traces of chloride in the suspected chlorate and to ensure that thiocyanate is absent.

**B. Reagents**

- (a) Sodium Nitrite Solution - 1% aqueous, Dissolve 0.5 g ACS Reagent Grade Sodium Nitrite in 50 mL high resistance deionized water.
- (b) Silver Nitrate Solution - 1% aqueous, Dissolve 1 gm ACS Reagent Grade  $\text{AgNO}_3$  in 100 mL of high resistance deionized water. Store in brown bottle.
- (c) Nitric Acid - 6N. dilute 20 mL ACS Reagent Grade  $\text{HNO}_3$  to 50 mL with high resistance deionized water.

**C. Determination**

Pipet 3-5 drops of a solution of suspected chlorate in water into a spot plate or vial, add 2-3 drops of 6N  $\text{HNO}_3$  and 1-2 drops of 1%  $\text{AgNO}_3$ . Note precipitation or turbidity. If insignificant, add 3-4 drops

1% NaNO<sub>2</sub> solution and 2-3 drops additional AgNO<sub>3</sub> solution. A white precipitate indicates ClO<sub>3</sub><sup>-</sup>. No precipitate is obtained with equivalent levels of ClO<sub>4</sub><sup>-</sup>. (CRM test 06 Apr 99)

D. **Detection Limit**

not determined

E. **Pertinent References**

1. Curtman, L.J. *Introduction to Semimicro Qualitative Chemical Analysis*  
The Macmillan Company New York, N.Y. 1950 p. 299

### Hypochlorite - Potassium Iodide Color Test

A. **Principle**

Hypochlorite can oxidize heavier halides to free halogens in acidic medium. Advantage can be taken of the high solubility of free halogens in halogenated solvents such as CCl<sub>4</sub> relative to water to concentrate liberated bromide or iodine and enhance visualization of a characteristic color. Levels of either bromide or iodide relative to one another could interfere by color masking.

B. **Reagents**

Potassium Iodide Solution - 0.5 M. Dissolve 8.301 g ACS Reagent Grade KI in 100 mL of high resistance deionized water.

Hydrochloric Acid - 6N. Dilute 25 mL ACS Reagent Grade HCl to 50 mL with high resistance deionized water.

C. **Determination**

Dissolve a small amount of the suspected solid in water or pipet 8-10 drops of a suspected hypochlorite solution into a 1 dr vial. Acidify with 2-3 drops of 6 N HCl. Add dropwise 4-5 drops of 0.5 M KI solution followed by 6-8 drops of CCl<sub>4</sub>. Shake for a few seconds and allow layers to separate. Observe presence of color in the CCl<sub>4</sub> layer. A pale to dark lavender color, depending on concentration, indicates hypochlorite.

D. **Detection Limit**

Not determined

E. **Pertinent References**

None

### Perchlorate in Water Extracts - Methylene Blue Spot Test

A. **Principle**

Perchlorates form violet precipitates with the methylene blue cation. With high levels of perchlorate, the lavender precipitate forms readily but, at lower concentrations, can be facilitated by addition of zinc sulfate and sodium nitrite. Only persulfate is reported to interfere but can be eliminated by boiling the sample prior to testing.

**B. Reagents**

(a) Methylene Blue - 0.03-0.05% aqueous solution

(b) Zinc Sulfate - saturated aqueous solution

(c) Sodium Nitrite - dissolve 13.80 g ACS Reagent Sodium Nitrite in 100 mL of high resistance deionized water.

**C. Determination**

Place 2-3 drops of water extract solution in a white plastic disposable spot plate. In an adjacent well, place an equivalent amount of water. To each plate, add 3-4 drops of methylene blue solution. Rapid formation of a violet precipitate indicates perchlorate. With lower concentrations, observe both wells, if the blue color in the test well fades and a small amount of dark precipitate forms, while the color of the water control well is unchanged, perchlorate is indicated. Alternatively, several drops of the test solution can be placed in a spot plate well, and 1-2 drops each of  $ZnSO_4$  and  $NaNO_2$  solutions added. A drop of the methylene blue solution is added and the color observed as previously described.

**D. Detection Limit**

Observed: 500 ppm (in-house 1989)

**E. Pertinent References**

1. Benedetti-Pichler, A.A. *Identification of Materials* Academic Press New York, N.Y. 1964 p. 391
2. Jungreis, E. *Spot Test Analysis Clinical, Forensic and Geochemical Applications* John Wiley & Sons New York, N.Y. 1985 p. 69

### **Nitrite in Water Extracts - Greiss Spot Test**

**A. Principle**

Nitrites react with primary amines in mildly acidic solutions to form diazonium cations. Further reactions produce highly colored azo dyes. These intensely colored products are visually apparent at low ppm levels. Chemical interferences, if any, are rare. Highly colored solutions may pose a problem but, with the extreme sensitivity of the test, can often be mitigated by dilution of the test sample.

**B. Reagents**

(a) Sulfanilic Acid Solution - Dissolve 1 g ACS Reagent Grade Sulfanilic Acid by warming in 100 mL of 30% Acetic Acid (aqueous).

(b) N-1-naphthylethylenediamine dihydrochloride Solution. Dissolve 1 gram of ACS Reagent Grade in 100 mL of 70/30 ethanol/acetic acid solution. Store in a brown glass bottle. Alternatively, a 0.1% aqueous solution, A.P.H.A. Cat. No. LC17550-7 is available from LabChem Inc. and has acceptable stability.

C. **Determination**

Pipet 4-5 drops of water extract solution to a ½ dr vial or 2-3 drops to a well in a white disposable spot plate. Add 1-2 drops of Sulfanilic Acid Solution followed by 1-2 drops of naphthylethylenediamine solution. An immediate rose to deep red color indicates nitrites. At low levels, color development may occur after standing a short time.

D. **Detection Limit**

10 ppm - immediate strong rose

5 ppm - distinct rose

2 ppm - distinct light rose in-house 29Aug97

reported: 0.01 ppm (as nitrous acid) (1)

E. **Pertinent References**

1. Feigl, F. and Anger, V. *Spot Tests in Inorganic Analysis*  
Elsevier Publishing Co. New York, N.Y. 1972 pp. 364-365

### Nitrate in Water Extracts - Modified Greiss Test

A. **Principle**

Nitrites react with primary amines in mildly acidic solutions to form diazonium cations but nitrates undergo no such reactions. Once produced, the diazonium cations react further to produce colored azo dyes. With an active metal such as zinc, in an acid medium, nitrates can be reduced to nitrites which then react to form the azo dyes. Interferences, if any, are rare. Because of the intensity of the color, detection of small amounts of nitrate in the presence of nitrites is difficult. Some indication of the presence of nitrates may be gained by diluting the sample and testing two equivalent portions side-by-side in a spot plate. Zinc is added to one and not the other. If the zinc containing sample exhibits more intense color, it may contain nitrates in addition to nitrites.

B. **Reagents**

- (a) Sulfanilic Acid Solution - Dissolve 1 g ACS Reagent Grade Sulfanilic Acid by warming in 100 mL of 30% Acetic Acid (aqueous).
- (b) N-1-naphthylethylenediamine dihydrochloride Solution. Dissolve 1 gram of ACS Reagent Grade in 100 mL of 70/30 ethanol/acetic acid solution. Store in a brown glass bottle. Alternatively, a 0.1% aqueous solution, A.P.H.A. Cat. No. LC17550-7 is available from LabChem Inc. and has acceptable stability.
- (c) Zinc powder, fine

C. **Determination**

Pipet 4-5 drops of water extract solution to a ½ dr vial or 2-3 drops to a well in a white disposable spot plate. Add 1-2 drops of Sulfanilic Acid Solution followed by 1-2 drops of naphthylethylenediamine solution. If little color develops, add a small amount (a few mg) of zinc powder. A rose to deep red color developing in the vicinity of the zinc powder indicates nitrates. With high levels, color develops rapidly but low levels may require a minute or more for color development to become apparent.

D. **Detection Limit**

0.05 ppm (as nitric acid) [1]

E. **Pertinent References**

1. Feigl, F. and Anger, V. *Spot Tests in Inorganic Analysis* Elsevier Publishing Co. New York, N.Y. 1972 pp. 363
2. Jungreis, E. *Spot Test Analysis Clinical, Forensic and Geochemical Applications* John Wiley & Sons New York, N.Y. 1985 pp. 207-209

### Oxidizer Screening in Water Extracts - Diphenylamine Test

A. **Principle**

Several strong or moderate oxidizing compounds produce a color with diphenylamine in concentrated sulfuric acid. Oxidation of diphenylamine to diphenylbenzidine results in production of the blue quinoid immonium ion. Development of color with DPA is a useful screening test for nitrate, nitrite, or chlorate. In addition to commonly encountered oxidizers, other compounds reacting with DPA include concentrated solutions of  $\text{FeCl}_3$  while  $\text{ClO}_4^-$  produces no color. The color developed and its persistence are functions of the species and also related to concentration. *Because it is non-specific, the test is presumptive for oxidizers and useful for sample screening only.*

B. **Reagents**

- (a) Sulfuric Acid, 18M - A.C.S. Reagent Grade
- (b) Diphenylamine, 1 gram dissolved in 100 mL concentrated sulfuric acid

Note: *Use with ventilation and handle to prevent spills.*

C. **Determination**

Pipet 1-2 drops of water extract solution into a white disposable plastic spot plate. Add 1-2 drops of diphenylamine solution. Observe color. An immediate and permanent ultramarine indicates  $\text{NO}_3^-$ . A similar color is obtained with relatively concentrated solutions of  $\text{FeCl}_3$ . Immediate blue colors are produced by  $\text{ClO}_3^-$  and  $\text{NO}_2^-$  but color from the latter fades rapidly and in about 1 minute is yellow green. At low levels, color development may occur after standing a short time. Similar reactions may also be observed with other less frequently encountered oxidizers.

D. **Detection Limit**

Varies: Not Applicable

E. **Pertinent References**

1. Feigl, F. and Anger, V. *Spot Tests in Inorganic Analysis* Elsevier Publishing Co. New York, N.Y. 1972 pp. 359-360
2. Jungreis, E. *Spot Test Analysis Clinical, Forensic and Geochemical Applications* John Wiley & Sons New York, N.Y. 1985 pp. 64-66

### Sulfate in Water Extracts - Barium Chloride Spot Test

A. **Principle**

Barium Chloride produces turbidity in solution at ppm levels and forms a white precipitate with higher levels of sulfate in acidic medium. Common barium salts of other anions are soluble in acid and do not interfere. The test is specific [1]

B. **Reagents**

Barium Chloride Solution - 5% aqueous, Dissolve 5 g ACS Reagent Grade  $\text{BaCl}_2$  in 100 mL of high resistance deionized water.

Hydrochloric Acid - 6N. Dilute 25 mL ACS Reagent Grade HCl to 50 mL with high resistance deionized water.

C. **Determination**

Pipet 4-5 drops of water extract solution to a  $\frac{1}{2}$  dr vial. Acidify with 1-2 drops of 6 N HCl. Add dropwise 3-4 drops of 5%  $\text{BaCl}_2$  solution, shake and observe turbidity or precipitate. If no initial turbidity or precipitate is observed, set sample aside and recheck in 10-15 minutes. With relatively low levels of sulfate, precipitate may form slowly. Heating of the sample may also enhance precipitate formation.

D. **Detection Limit**

Not Determined

E. **Pertinent References**

1. Benedetti-Pichler, A.A. *Identification of Materials*  
Academic Press New York, N.Y. 1964 p. 381

### Sulfide in Water - Silver Nitrate Spot Test

A. **Principle**

Silver Nitrate gives a brown/black precipitate with low levels of sulfide (or hydrosulfide) in dilute acidic medium. Aqueous sulfide(s) are readily oxidized to sulfate by heating with dilute  $\text{HNO}_3$ . In acidic medium, silver sulfate is soluble so no precipitate is observed.

B. **Reagents**

(a) Silver Nitrate Solution - 1% aqueous, Dissolve 1 gm ACS Reagent Grade  $\text{AgNO}_3$  in 100 mL of high resistance deionized water. Store in brown bottle.

(b) Nitric Acid - 6N. dilute 20 mL ACS Reagent Grade  $\text{HNO}_3$  to 50 mL with high resistance deionized water.

C. **Determination**

Pipet 4-5 drops of water extract solution to a  $\frac{1}{2}$  dr vial. Acidify with 1-2 drops of 6 N  $\text{HNO}_3$ . Add dropwise 2-3 drops of 1%  $\text{AgNO}_3$  solution, shake and observe turbidity or precipitate. If turbidity is observed which darkens in a few seconds, continue observation. If the precipitate becomes brown/black, test a fresh sample of the original extract, acidify with 4-5 drops of 6N  $\text{HNO}_3$ , heat to boiling for a few seconds, cool and retest with  $\text{AgNO}_3$ . A white precipitate of  $\text{AgCl}$  indicates that both



sulfide(s) and chloride are present in the sample. No precipitate indicates sulfide(s) only

D. **Detection Limit**

Not determined

E. **Pertinent References**

None

### Sulfur Spot Test

A. **Principle**

Crystalline free sulfur is extracted from the questioned material with pyridine. The color observed will vary from blue or green for low concentrations to brown for high concentrations. Interferences include water and aluminum. Hydrocarbons and CS<sub>2</sub> inhibit color formation. Polyhalogenated hydrocarbons, CHCl<sub>3</sub>, Cl<sub>2</sub>, Br<sub>2</sub>, and I<sub>2</sub> also interfere.

B. **Reagents**

(a) Pyridine

(b) 10% NaOH – dissolve 10g NaOH in 100mL of water.

C. **Determination**

Place a piece of material suspected to contain sulfur in small vial or test tube (remember to run both positive and negative controls). Add several drops of pyridine and gently warm. Remove the vial from heat and add approximately one-tenth its volume of sodium hydroxide solution. The formation of a blue, green or brown color indicates the presence of sulfur. The color will begin to fade after a few moments.

If no particles of sulfur are visible or if the sample contains powdered metal, wash the dry solid with pyridine, collect the filtrate and test with sodium hydroxide as above. A disposable glass pipet with glass wool plugs can be used as a filtering mechanism.

D. **Detection Limit**

Not determined. According to Lange it is detectible at a concentration of 1:300,000 in pyridine.

E. **Pertinent References**

1. Lange, E. , *Handbook of Chemistry, 9<sup>th</sup> Edition*, Handbook Publishers, Inc., 1956 p. 981-982
2. Meyers R., "A Systematic Approach to the Forensic Examination of Flash Powders", *Journal of Forensic Sciences*, Vol 23, #1, 1978
3. Jungreis, E. *Spot Test Analysis Clinical, Forensic and Geochemical Applications* John Wiley & Sons New York, N.Y. 1985 p. 69

## Thiocyanate in Water - Ferric Chloride Spot Test

### A. Principle

Thiocyanate and Chloride both give white precipitates with  $\text{AgNO}_3$  in acidic medium. Ferric Chloride solution gives a rose to blood red color with thiocyanate in slightly acidic or neutral aqueous solutions. Color hue and intensity are dependent on  $\text{SCN}^-$  concentration.

### B. Reagents

Ferric Chloride Solution - 10% aqueous. Dissolve 1 g ACS Reagent Grade  $\text{FeCl}_3$  in 10 mL of high resistance deionized water. Store in brown bottle.

### C. Determination

Pipet 1-2 drops of water extract solution to the well of a white disposable plastic spot plate. Add dropwise 2-3 drops of 10%  $\text{FeCl}_3$  solution, and observe color development. A faint rose color indicates low levels of  $\text{SCN}^-$  and an intense red, much higher levels. If the original extract solution is highly colored, traces of  $\text{SCN}^-$  may not be observed. Testing of lightly colored extracts can be facilitated by using, in an adjacent spot plate well, an equivalent amount of extract solution, e.g. 1-2 drops and adding water equivalent to the volume of the amount of  $\text{FeCl}_3$  solution used. Colors of the control and test samples are compared.

If desired, an additional sample is acidified with 4-5 drops of 6N  $\text{HNO}_3$  and heated to boiling for a few seconds. The cooled sample is then retested with  $\text{FeCl}_3$ , a clear yellow, rather than red, color is indicative of  $\text{SCN}^-$ . Similar results are obtained with oxidation of the  $\text{SCN}^-$  and testing with silver nitrate, i.e. no white precipitate.

### D. Detection Limit

Not determined

### E. Pertinent References

1. Curtman, L.J. *Semimicro Qualitative Chemical Analysis*  
Macmillan Co. New York, N.Y. 1954 p. 297
2. Benedetti-Pichler, A.A. *Identification of Materials*  
Academic Press New York, N.Y. 1964 p. 361

## Aluminum Spot Test - Morin Reagent

### A. Principle

Aluminum salts and aluminum in neutral or slightly acidic solution reacts with Morin Reagent to form a complex that produces intense green fluorescence under UV light. Interferences include free and salts of: beryllium, indium, gallium, thorium, scandium and zirconium.

### B. Reagents

Morin Reagent (3,5,7,2',4'-pentahydroxyflavanone), saturated solution in alcohol  
10% NaOH (aq)  
Dilute acid

C. **Determination**

Add 2 drops of 10% NaOH to a dark well plate. Add small amount of suspected aluminum. The alkaline solution digests the aluminum with the evolution of hydrogen gas. This digestion may be noted macroscopically and/or microscopically. Add diluted acid to aqueous test solution to make the solution slightly acidic. Add 2 drops of Morin Reagent and observe under UV light. A fluorescent green color is a positive test result.

D. **Detection Limit**

0.2 µg/mL aluminum

E. **Pertinent References**

1. Houghton, Rick *Field Confirmation Testing for Suspicious Substances*, CRC Press Taylor & Francis Group, 2009, pages 277-278.
2. Jungreis, Ervin *Spot Test Analysis: Clinical, Environmental, Forensic, and Geochemical Applications, 2<sup>nd</sup> Edition*, Wiley-Interscience, 1997, page 89.
3. Paul, Armine and Gibson, John *Qualitative Analysis for Aluminum*, Journal of Chemical Education, 1962, 39(8), page 398.