SER: Saliva- Radial Diffusion for Amylase

Principle

Amylase is an enzyme present in saliva at elevated levels and in other body fluids (such as semen, vaginal fluid, urine) at lower levels. Amylase hydrolyzes starch, which is contained in the radial diffusion plate gel medium.

As the sample extracts diffuse through the gel, starch is hydrolyzed in an area proportional to the concentration of active amylase in the sample extract. An iodine solution will stain areas of unhydrolyzed starch blue while hydrolyzed starch remains unstained.

The amylase radial diffusion test is used to approximate the level of amylase in a sample. While amylase is present in many biological fluids, elevated levels of amylase may indicate the presence of saliva. Comparing the results to known concentrations of amylase may allow the analyst to report a finding of an "elevated level" of amylase.

Equipment and supplies

This procedure uses the following equipment and supplies:

- centrifuge
- oven
- plastic Petri dishes
- disposable Pasteur pipettes with tip openings of approximately 1.5 mm diameter
- pipette
- pipette tips
- microcentrifuge tubes

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Buffers

This procedure uses one of the following buffers:

- Amylase Diffusion Gel Buffer
  - Dissolve the contents of the *Amylase Diffusion Gel Buffer* pre-mix media in 500 mL of purified water. Check and adjust to pH 6.9. Store buffer solution at 5°C ± 5°C.
- Alternate Preparation of Gel Buffer
  - 2.7 g $NaH_2PO_4 \cdot H_2O$
  - 3.9 g Na_2HPO_4
  - 0.2 g NaCl
    - Dissolve in deionized water and bring the final volume up to 500 mL. Check the pH and adjust to 6.9.

Reagents

This procedure uses the following reagents:

- α-amylase standard (1 U/µL)
  - Reconstitute lyophilized amylase in enough deionized water to yield a one unit per µl solution (e.g. reconstitute a 500U bottle with 500 µL DI water). Aliquot the reconstituted standard and store at -20°C ± 5°C.
- Stock iodine solution
  - 1.65 g potassium iodide
  - 2.5 g iodine
    - Dissolve in 30 mL warm deionized water and filter.
- Working iodine solution
  - Mix 1 milliliter of the *Stock iodine solution* with 99 mL of deionized water.

Quality control

The amylase standard must be quality control tested. Records documenting the preparation and quality control testing will be kept in the *Biology Quality Control Log Book*.

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Support medium

To prepare the support medium, dissolve 0.15 g of SERI type EA agarose in 15 mL of *Gel Buffer* (see above) using heat. Pour into a plastic Petri dish.

NOTE: If SERI EA agarose is not available, dissolve 1.5 grams Sigma Type II agarose (or agarose of similar EEO) and 0.1 gram soluble starch in 15 mL of Gel Buffer.

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Controls

The following controls must be included with each test:

A negative control (saline blank)
A positive control of either:

- Amylase standard (1U/uL); or
- Known saliva sample

Positive controls are used in the following dilutions:

- 1:100 dilution of positive control in saline
- 1:500 dilution of positive control in saline

Sample preparation

The stain cutting (approximately 5 mm x 5 mm) or swab (approximately one quarter) should be extracted in 50 µL of saline for approximately 30 minutes and all cellular material pelleted by centrifugation. This procedure uses the resulting supernatant.

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Procedure

Use the following procedure to perform amylase radial diffusion.

Step	Action
1	Punch wells into the support medium using a Pasteur pipette.
2	Load 2 µL of the 1:100 positive control dilution, 1:500 positive control dilution, saline blank, and the samples into the appropriate wells.
3	Incubate in the oven at 40°C ± 5°C for approximately 16 hours.
4	Cover the surface of the support medium with the *Working iodine solution.* When the background turns blue, pour off the solution and rinse with water.
5	Measure the diameter of the clear circles surrounding the wells and record the results.

Results

Clear circles of the gel are a positive (+) indication for the presence of amylase.

An absence of clearing is a negative (-) indication for the presence of amylase.

Results are inconclusive and the assay should be repeated if:

- the 1:500 dilution shows no amylase activity
- amylase activity is detected in non-sample areas of the plate
- amylase activity is detected in the saline blank sample

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Interpretation

This is a test for the presence of amylase. Amylase levels in saliva are reported to be much higher than amylase levels in other body fluids. Perspiration, breast milk, and semen have been reported to contain elevated levels of amylase on rare occasions. Since amylase is not unique to saliva, this test is not a conclusive test for saliva.

An area of clearing measuring greater than or equal to the 1:100 dilution of the positive control is considered an elevated level of amylase activity and indicates the presence of saliva.

An area of clearing measuring less than the 1:100 dilution of the positive control is considered a low level of amylase activity and is an inconclusive result for saliva. When an area of clearing is smaller than the 1:100 dilution of positive control and difficult to measure, it can be reported as a trace level of activity and an inconclusive result for saliva.

The absence of clearing is a negative result for the presence of amylase.

NOTE: Mixtures of body fluids can have an additive effect on the levels of amylase present and some people have much higher levels of amylase in their body fluids than others.

The level of amylase activity may be used as a guide for selecting samples for DNA analysis as saliva typically contains epithelial cells. Samples with low or no amylase activity may still be suitable for further genetic testing.

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Reporting guidelines

General report wording describing the test:

The report may read:
"Amylase is an enzyme found at an elevated level in saliva and at a lower level in other body fluids."

1. Because the amylase test is not conclusive for saliva, an appropriate caveat should be included when clearing measuring greater than or equal to the 1:100 standard is observed:

   The report may read:
   "I detected an elevated level of amylase activity in [the sample] which may indicate the presence of saliva. However, perspiration, breast milk, and semen have been reported to contain elevated levels of amylase activity on rare occasions."

2. With the exception of a vaginal sample, when amylase is detected in combination with the presence of nucleated epithelial cell:

   The report may read:
   "I found nucleated epithelial cells and detected an elevated level of amylase activity in [the sample] which may indicate the presence of saliva."

3. If there are detectable levels of amylase with clearing below the 1:100 standard:

   The report may read:
   "I detected a low/trace level of amylase activity in [the sample]. A low/trace level is inconclusive for the presence of saliva."

4. When no amylase activity is observed:

   The report may read:
   "I did not detect any amylase activity in [the sample]."

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SER: Saliva- Radial Diffusion for Amylase, Continued

References

The following references were used in the development of this procedure:

- Basic Forensic Serology- CCI, January 2001
- *Forensic Examination of Sexual Assault Evidence Training Manual (Section 25)*, California Criminalistics Institute, California Department of Justice, 1992.
- *Source Book is Forensic Serology, Immunology & Biochemistry Section II- Identification of Saliva.*
- Kipps, A.E., and P.H. Whitehead, "The Significance of Amylase in Forensic Investigations of Body Fluids, "Forensic Science, vol6, 1975, pp. 137-144.
- Auvdel, Michael J., "Amylase Levels in Semen and Saliva Stains," Journal of Forensic Science, vol. 31, no. 2, April 1986.
- Blake, Edward T., and Sensabaugh, G.F., Differential Expression of Amylase Loci [Amy1 and Amy2] in Human Body Fluids and Secretions. Presented at the 46th Semiannual Seminar of the California Association of Criminalists, October, 1975.