Rapid Colorimetric Surface Protein Test: Determination of the Limit of Detection for AllerSnap

Application

AllerSnap is a quick and easy way to detect allergenic and non-allergenic protein residues left on a surface after cleaning. Simply swab a surface, release the reagent and incubate; if food residue containing protein is present the reagent will turn from green to purple. The color change provides a semi-quantitative measure for such residues. The more protein present, the quicker the color change to purple and the darker the color. AllerSnap quickly measures the contamination of a surface, allowing immediate corrective action to be taken if necessary.

Principle

The test is based on the principle of the Biuret reaction where, under alkaline conditions, the copper (II) ions form a complex with the peptide bonds of proteins and are reduced to copper (I) ions. Bicinchoninic acid (BCA), under alkaline conditions, is a highly sensitive, stable and specific reagent for copper (I) ions, forming a purple complex when bound to copper (I) ions. The chromogen formed can be assessed visually with the AllerSnap device.

$$\text{Protein} + Cu^{2+} \xrightarrow{OH^-} \text{Protein-}Cu^{+} \xrightarrow{+\ \text{BCA}} \text{Purple complex}$$

AllerSnap detects peptide bonds, which are present in all proteins, so it does not discriminate between the types or sizes of proteins detected.

Performance

The reaction is time-dependent, i.e. the color develops with time, therefore it is important to record color change up to 30 minutes when incubated at 37°C or up to 15 minutes when incubated at 55°C; any color change after this set time should be disregarded. A positive result for protein residue can be noted as soon as any color change is observed.

Precision of the reaction is temperature-dependent, therefore it is important to allow the devices to equilibrate to ambient room temperature (15 - 25°C) if they have been stored at a different temperature. Following such equilibration, the devices can be then incubated according to instructions.

False Positives

This test also detects other substances capable of reducing the copper (II) ions to copper (I) ions, such as reducing sugars (glucose) and uric acid. Other strong reducing materials such as ascorbic acid (present in some fruit juices) or tannin (present in tea) may also give a positive result with AllerSnap.

Sensitivity

3 µg 30 minutes at 37 °C or 15 minutes at 55 °C.

The chart below shows the color change associated certain dilutions of protein derived allergens. (µg/10µl, where 1 µg/ml equals 1 ppm)

Levels	Detection	Color Change	Result
1	< 3 µg	Green	Pass
2	3- 5 µg	Greyish Green	Caution
3	6 – 10 µg	Grey	Fail
4	11-25 µg	Light Purple	Fail
5	≥ 26 µg	Dark Purple	Fail

Evaluation

Determining detection at 37 °C for 30 minutes or 55 °C for 15 minutes

Purpose

Determine the detection limits and linearity of response of the AllerSnap presented with Bovine Serum Albumin (BSA) Protein. Incubation at 37°C and 55°C evaluated.

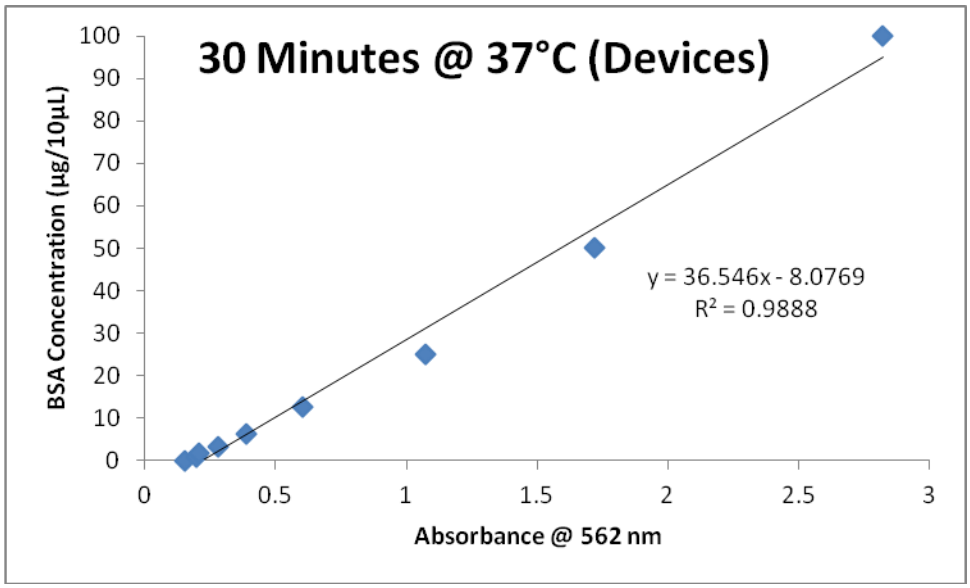
Materials and Methods

- Incubators specific for such devices are set at 37 °C and 55 °C
- In-date AllerSnap devices are used throughout
- In addition to color observations, the absorbance (A) of the BCA-Cu^{1+} complex was recorded at 562nm
- Concurrent determinations are performed in glass test-tubes using the same reagents and volumes as the AllerSnap configuration

Results

I. Protein detection at 37 °C, 30 minute device incubation

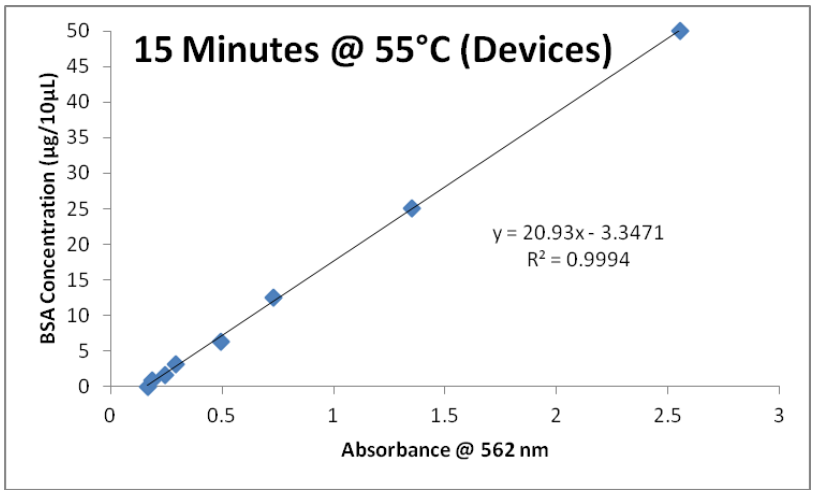
Observation		
µg of BSA	**A (562)**	**Color**
0.00	0.155	Green
0.78	0.196	Green
1.56	0.207	Green
3.12	0.282	Greyish Green
6.25	0.387	Grey
12.50	0.602	Light Purple
25.00	1.071	Light Purple
50.00	1.719	Dark Purple
100.00	2.821	Dark Purple



Graph 1: Standard curve for BSA in devices incubated 30 minutes at 37 °C shows a 98.88% correlation.

II. Protein detection at 55 °C, 15 minute device incubation

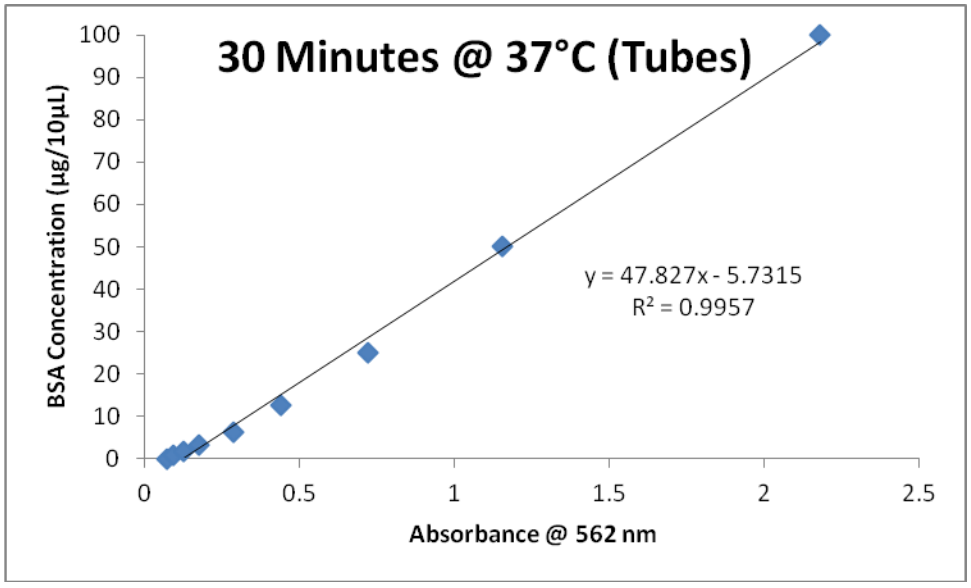
Observation		
µg of BSA	**A (562)**	**Color**
0.00	0.167	Green
0.78	0.187	Green
1.56	0.245	Green
3.12	0.293	Grey
6.25	0.497	Grey
12.5	0.728	Light purple
25.0	1.350	Light purple
50.00	2.553	Dark purple
100.00	>3.000	Dark purple



Graph 2: Standard curve for BSA in devices incubated 30 minutes at 55 °C shows a 99.94 % correlation.

III. Protein detection at 37 °C, 30 minute incubation in test-tubes.

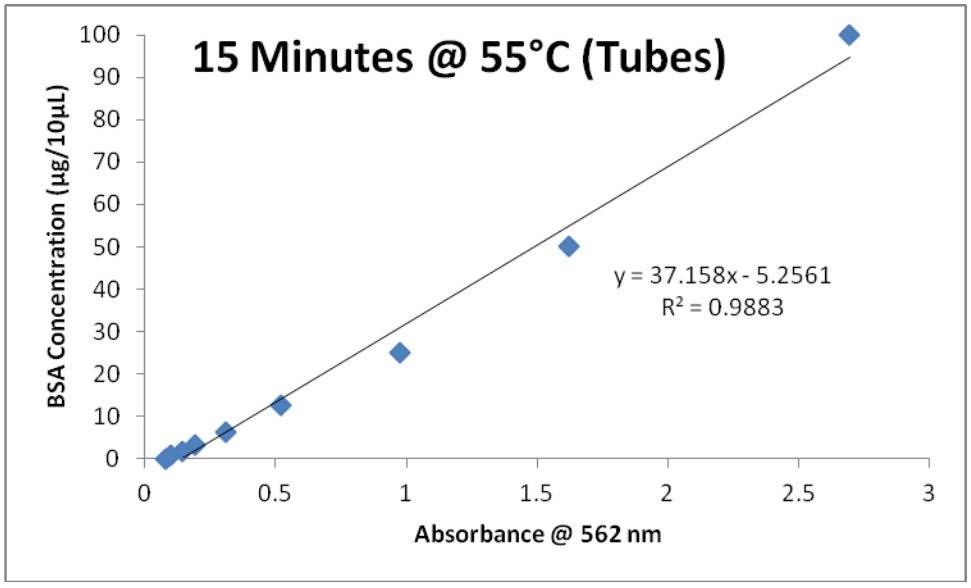
Observation		
µg of BSA	**A (562)**	**Color**
0.00	0.073	Green
0.78	0.092	Green
1.56	0.124	Green
3.12	0.173	Greyish green
6.25	0.286	Grey
12.50	0.440	Light purple
25.00	0.720	Light purple
50.00	1.156	Dark purple
100.00	2.180	Dark purple



Graph 3: Standard curve for BSA in glass test-tubes incubated 30 minutes at 37 °C shows a 99.57% correlation.

IV. Protein detection at 55 °C, 15 minute incubation in test-tubes.

Observation		
µg of BSA	**A (562)**	**Color**
0.00	0.079	Green
0.78	0.101	Green
1.56	0.142	Green
3.12	0.193	Grey
6.25	0.311	Grey
12.50	0.518	Light purple
25.00	0.977	Light purple
50.00	1.620	Dark purple
100.00	2.695	Dark purple



Graph 4: Standard curve for BSA in glass test-tubes incubated 15 minutes at 55 °C shows a 98.83% correlation.

Observations

- There is good correlation between protein determination at 37 °C and 55 °C
- The detection limit at is 3 µg of BSA protein
- Elevated temperatures provide faster results
- The devices can be used to detect allergenic and non-allergenic proteins