

Pro-tect[®] M for Monitoring Cleaning in Hospital Sterile Services Washing Equipment

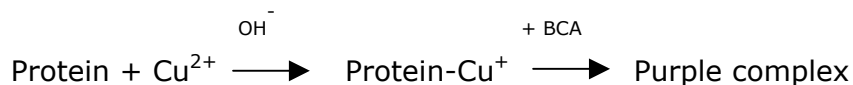
The need to carry out mandatory testing of washer disinfectors is laid out in the HTM2030 document, notably paragraphs 10.37 to 10.51. It is recommended that items processed in the washer disinfectors in hospital Sterile Service departments be tested for 'residual soil' after cleaning at least once a week although internal quality assurance policies often require this testing to be done on a more frequent basis.

Standard testing has traditionally used protein detection procedures based on ninhydrin reagents but this technique is laborious, requiring the use of 110°C incubator, and necessitates making up the required reagents.

However the regulations also allow for tests based on other protein detection methods (paragraph 10.49), notably the Biuret reaction; this forms the basis of the reagents used in Pro-tect[®] M, where in the presence of protein the chemicals react to form deep purple colour. Pro-tect[®] M is a sensitive and easy to use 'single shot' protein test from Biotrace which can be used to monitor the cleaning efficacy of hospital washing equipment.

Principle of the test

The Pro-tect[®] M test is based on the patented (Numa et al 1999) Konica enhanced Biuret colour change chemistry. Under alkaline conditions copper ions (Cu^{2+}) form a complex with (protein) peptide bonds and are reduced to copper Cu^+ . Bicinchoninic acid (BCA) under alkaline conditions is a highly sensitive, stable (no need for refrigeration) and specific reagent for Cu^+ and leads to the formation of the purple complex. The chromogen, once formed, can be assessed visually (as with the Pro-tect[®] M devices) or assayed with a Spectrophotometer (Absorbance at 562nm).



Paragraph 10.47 (HTM 2030) states that the protein test should be able to detect <2mg/m² determined as arginine, or better. If it were assumed that a minimum 10cm² sample size is swabbed this would make the required sensitivity of the test 2ug of protein.

The limit of detection of Pro-tect[®] M using our standard 10 minute incubation step was experimentally determined as 50 to 60ug with a 100cm² swab area (Bovine Serum Albumin).

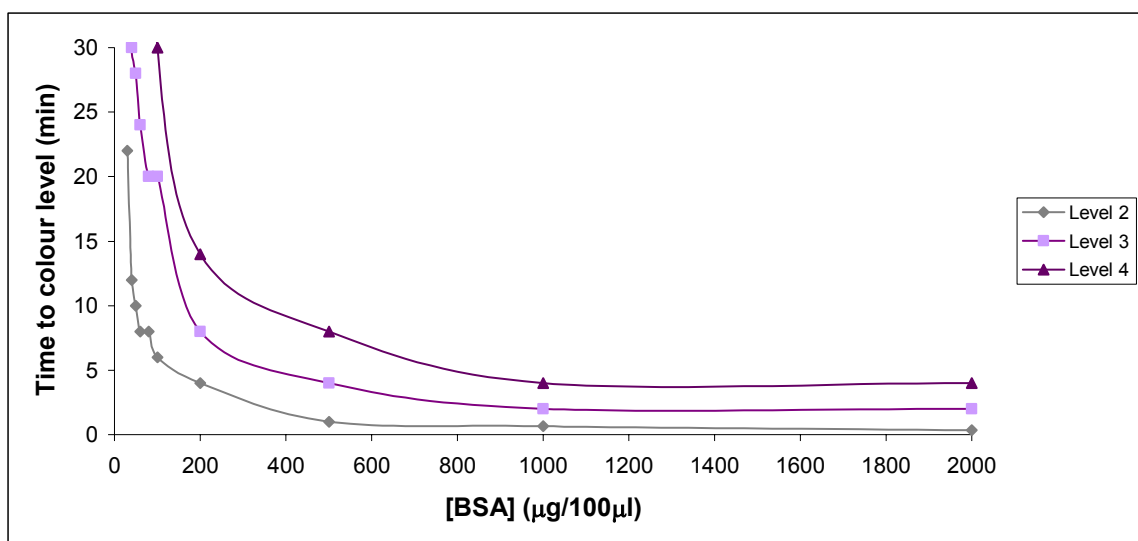
Note: Pro-tect[®] M detects the peptide bonds of proteins and therefore will not detect individual amino acids such as arginine.

Table 1: Detection of protein (Bovine Serum Albumin or BSA). Detection on the swab and spread on a 10x10 cm surface area (tested wet and dry) were compared.

Amount of protein/100 μ l	Colour level (1-4)		
	On Swab	Wet Surface	Dry Surface
10000 μ g	4	4	4
5000 μ g	4	4	4
2500 μ g	4	3/4	3/4
1250 μ g	4	3/4	3/4
625 μ g	4	3/4	3
312 μ g	3/4	3	3
156 μ g	3	3	2/3
100 μ g	3	2	2
80 μ g	2/3	2	2
60 μ g	2	2	1/2
50 μ g	2	1/2	1
40 μ g	1/2	1	1
0 μ g (water only)	1	1	1

Additional work was then carried out on the test kinetics and this showed that the rate of reaction was dependent on the incubation temperature of the assay as well as the amount of protein present.

Fig 1 The time taken to reach the different colour levels for a range of protein (BSA) concentrations



The above data demonstrates that a direct relationship exists between sensitivity and rate of the chemical reaction.

In order to increase the sensitivity of the test further experimentation was carried out to lower the limit of detection by optimising the temperature and time assay parameters.

Sensitivity of the test procedure was examined at 37°C over time.

BSA (µg/100µl) Time (min)	1000	500	200	100	50	30	20	10	5	2.5	1.25	0 R 0 water
0	1/2 1/2	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1	1 1
2	4 4	3/4 3/4	3 3	2/3 2/3	2 2/3	2 2	2 1/2	1 1	1 1	1 1	1 1	1 1
4	4 4	4 4	3/4 3/4	3 3	2/3 3	2 2P	2 2	1/2 1/2	1 1	1 1	1 1	1 1
6		4 4	4 4	3 3/4	3 3	2/3 2/3	2/3 2P	1/2 1/2	1 1	1 1	1 1	1 1
8			4 4	3 3/4	3 3	2/3 2/3	2/3 2P	1/2 1/2	1 1/2	1 1	1 1	1 1
10				3/4 4	3 3	3 3	2/3 2/3	1/2 1/2	1 1/2	1 1	1 1	1 1
12				4 4	3 3	3 3	3 2/3	2 2	1 1/2	1 1	1 1	1 1
14				4 4	3 3	3 3	3 3	2 2	1/2 2	1 1	1 1	1 1
16					3 3	3 3	3 3	2P 2P	1/2 2	1 1	1 1	1 1
18					3 3	3 3	3 3	2P 2P	1/2P 2P	1/2 1/2	1 1	1 1
20					3/4 3/4	3 3	3 3	2P 2P	1/2P 2P	1/2 1/2	1 1	1 1
22					3/4 3/4	3 3	3 3	2/3 2/3	1/2P 2P	1/2 1/2	1 1	1 1
24					3/4 3/4	3 3	3 3	2/3 2/3	1/2P 2P	1/2 1/2	1 1	1 1
26					3/4 3/4	3/4 3	3 3	2/3 2/3	1/2P 2P	1/2 1/2	1 1	1 1
28					3/4 3/4	3/4 3	3 3	2/3 2/3	1/2P 2P	1/2 1/2	1 1	1 1
30					3/4 3/4	3/4 3	3 3	2/3 2/3	1/2P 2/3	1/2 1/2	1 1	1 1
35					3/4 4	3/4 3/4	3 3	3 3	1/2P 2/3	1/2 1/2	1G 1G	1 1
40					4 4	3/4 3/4	3 3/4	3 3	2P 2/3	2 2	1G 1G	1 1
45					4 4	3/4 3/4	3 3/4	3 3	2P 3	2 2	1/2 1/2	1 1
50						4 3/4	3/4 3/4	3 3	2P 3	2 2P	1/2 1/2	1 1
55						4 3/4	3/4 3/4	3 3	2P 3	2P 2P	1/2P 1/2	1G 1G
60						4 3/4	3/4 3/4	3 3	2P 3	2P 2P	1/2P 1/2P	1G* 1G*
70						4 4	3/4 3/4	3 3	2/3 3	2P 2P	2 2	1G* 1G*
80						4 4	3/4 4	3 3	2/3 3	2P 2P	2 2P	1/2 1/2
90							3/4 4	3 3	2/3 3	2/3 2P	2 2P	1/2 1/2
100							4 4	3 3	2/3 3	2/3 2/3	2 2P	2 2
110							4 4	3 3	3 2/3	2/3 2/3	2P 2/3	2 2
120								3 3	3 3	2/3 3	2/3 2/3	2 2P

* solution starting to change colour

Key:

1	green
1/2	green-grey solution
1/2P	green-grey solution and pale purple swab
2	grey solution
2P	grey solution and pale purple swab
2/3	greyish purple solution
3	light purple solution
3/4	mid purple solution
4	dark purple solution

Conclusion

It can be observed that lengthening the test incubation time substantially increased the assay sensitivity by allowing the chemical reaction to continue over time. The rate of this colour change reaction was also observed to increase when carried out at 37°C (compared to ambient temperature).

The 2.5 µg/100µl sample could be detected (clearly different to control) after 40 min at 37°C. The 1.25 µg/100µl sample gave a clear positive (level 2) after 70 min and was different to the control at 45 min, although the colour change (level 1/2) at this time was not distinct enough at this stage to give a clear positive. The control swabs (no addition of protein) started to change to a grey colour after 60 minutes.

It is recommended that, as a protein test for washer disinfectors, Pro-tect® M is used to swab a >10cm² area and the device is then incubated at 37°C for 45 minutes. This provides a sensitivity of ~ 2mg/m² protein (as BSA).