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A test for the assessment of disinfectant/sanitizers used for napkin storage

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SUMMARY

A test is described for assessing the effect of hypochlorites in reducing the bacterial load on soiled napkins during storage, between removal from the infant and the laundering.

INTRODUCTION

Napkin storage products are specially designed to improve environmental safety in the home. They are practical aids for that part of the infant's daily routine, which involves the changing of up to 8 napkins per 24 h, and as it would be impracticable to wash each of these 8 napkins separately they must be stored till laundering.

The napkins, normally urea soaked, may also carry a bacterial load from faecal soil, even if the bulk of the deposit has been sluiced off. It is preferable therefore that the napkins are stored in a solution which is actively anti-bacterial to prevent risks of environmental contamination, and which reduces production of malodours, rather than in a non-antimicrobial, highly perfumed solution, which is just deodorizing. The antibacterial solution must have special characteristics, as it must kill bacteria added with napkin material, in the presence of large quantities of urea. Inactivation by urea of various antibacterials, notably hypochlorites, is well reported. If the faecally soiled napkin is placed in a soak bucket after urea soaked napkins, the activity of the solution may have been chemically destroyed and the soak bucket would become a potential hazard, due to surviving organisms. In addition the napkin material can adsorb various antibacterials, notably quaternary ammonium compounds, inactivating them and this would also result in contaminated soak water if the bacterial insult is not on the first nappy.

Obviously a test to evaluate the efficacy of actives in a napkin storage product must be specific, as it must take into consideration serial insults on the solution by urea, napkin material and bacteria.

Official methods do exist for testing disinfectant and sanitizing products, which were investigated in conjunction with the development of the Serial Urea Test Method. Among those studied were the Kelsey–Sykes test (Kelsey & Sykes, 1969), the Mallmann Carrier Test (Mallmann, & Hanes, 1945), the Association of Official

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Agricultural Chemists Available Chlorine Test (A. O. A. $CAvCl_2$), (A.O.A.C. Official Methods 10th Edition) and the Weber & Black Test (Weber & Black, 1948). These four are used routinely for assessments as defined by their protocols, but they are not considered applicable to the testing of a napkin storage product as none tests the antibacterial activity of a solution in the presence of multiple insults by chemicals similar to urea, nor considers the competition for antimicrobial agent between fabric and bacteria.

The Mallmann carrier test, for example, allows for only one bacterial insult and one chemical insult. It is not rigorous enough for a napkin storage product. The A.O.A.C. $Av.Cl_2$ test is for quick acting surface disinfectants, as is the Weber & Black test, which only differs in its consideration of activity in the presence of hard water. Lastly the Kelsey–Sykes test is not recommended for hypochlorites under dirty conditions, owing to inactivation of the germicide by organic matter (private communication with Dr D. Coates and in Press *Pharm. J.*).

In summary, a satisfactory test must take into account insults from a solution of urea, bacteria and napkin material over a period of hours, and must allow for an assessment of the activity of the antimicrobial, whether the faecally soiled napkin with the bacterial load is added first or at any stage up to eight of the day's napkins. We now describe such a test, the results of which also indicate at what stage any antimicrobial solution will be inactivated and provide evidence as to the cause of this reduction of activity.

MATERIALS AND METHODS

Serial urea insult test

Materials

The organism used in the test was *Escherichia coli* (*E. coli*) NCTC 9701 maintained on Nutrient Agar (Oxoid Blood Agar Base No. 2) subcultured into Oxoid Nutrient Broth No. 2 and grown overnight at 37 °C. 0·1 ml of broth culture was used to inoculate each of two 250 ml conical flasks containing 100 ml Nutrient Broth plus four napkin swatches. These were incubated overnight at 37 °C on a reciprocating shaker in a water bath.

Napkin swatches were cut from new cotton terry-towelling napkins, discarding double sewn edges. Each napkin swatch was 57 mm square; this size of napkin swatch carries approximately 1/10th of the inoculum which is present on a faecally soiled napkin after rinsing off the faecal soil; is technically easy to manipulate, and allows good aeration of the broth culture. The volume of exhausted nutrient broth carried by the swatch was felt to be sufficient to simulate the protein load carried by a faecally soiled napkin.

The neutralizer solution was 0·1 % Analar sodium thiosulphate in distilled water distributed in 9 ml volumes into universal bottles.

Urea solution was made using Analar reagent at 3 % in distilled water. To 500 ml of this solution 5 ml of 1/1000 overnight culture of *E. coli* NCTC 9701 was added. This resulted in a low inoculum of 10^3–10^4 per ml to simulate the relatively low inoculum found on a wet, unsoiled nappy.

The plating medium was Oxoid Blood Agar Base No. 2; the plates were dried in a warm air oven before inoculation.

The media and neutralizer solutions were sterilized at 121 °C (15 lb/in^2) in an Astell No. 6 electric autoclave for 15 min.

The experimental conditions were chosen so that the volumes were approximately 1/10 of those found under in-use conditions.

The inoculum carried by the napkin swatch results in 10^7–10^8 organisms per ml of soak solution. Burn, Lamb, Abbott & Watkinson (1969) reported counts of between 10^4 and 10^7 per ml in in-home samples.

Method

Counting technique

At half-hourly intervals during the experiment 0·5 ml samples of the contaminated neutralizer solutions are transferred to the surface of a dried plate and spread over the surface with the pipette tip. For the samples from the water control jar a full series of tenfold dilutions to 10^{-6} is used to give an accurate colony count at the beginning and end of the experiment.

Experimental details

A 500 ml volume of the napkin-storage preparation under test, prepared according to manufacturer's directions, is placed in each of seven 1000 ml capacity clean glass jars, and 500 ml of tap water is placed in a similar jar (jar 8) to act as control.

At time 0 the first urea insult is carried out, 7 ml of the urea solution being added with stirring to each jar. At the same time a contaminated napkin swatch, drained of excess broth, is added with stirring to jar 1 and to the control jar.

At time 28–30 min a 1 ml sample is taken from each jar and transferred to a bottle of neutralizer solution, mixed, and left standing before plating out when the second urea insult is completed. This, at 30 min, is a replica of the first urea insult at time 0. At the same time a contaminated swatch is added to jar 2.

At 58–60 min 1 ml samples are taken for addition to neutralizer before counting as before. A third urea insult is completed, and jar 3 receives a napkin swatch. The procedure is repeated at 30 min intervals, with urea insults to all jars and napkin insults to jar 4 at 1½ h, jar 5 at 2 h, and so on. Sampling and plating is continued till 3½ h, and at this time the second full colony count on the water control is carried out. Two hours after the last inoculum (or whatever is the manufacturer's minimum recommended contact time) during which there are no further urea or napkin insults, the sampling and plating procedures are repeated and a third full colony count on the water control is carried out. The plates of nutrient agar for the colony counts are incubated at 37 °C for 24 h.

These sampling and plating procedures allow accurate assessment of survivors/ml between 6×10^2 and 6×10^3, and approximate numbers down to 20 and up to 10^4. Any plate with more than 500 colonies was recorded as being too numerous to count (TNTC), and in such cases survivors/ml are reported as $> 10^4$.

RESULTS

Table 1 is representative of the pattern of results obtained by us in a number of experimental runs with our present product. The recoveries from jars 4 and 5 after 1½ and 2 h respectively are consistently higher than recovery from other jars at similar times after bacterial insult but are within the pass limits of the test. This low spot in the release-curve is a useful 'attack point' in exhaustive comparative studies, where a soiled nappy may be added at 2½ or 3 h.

Table 1. *Total colony counts/ml in the serial urea insult test, using a formulation giving continuous available chlorine release*

Time (h)	Jar no. 1	2	3	4	5	6	7	8
0	*NC	NC	NC	NC	NC	NC	NC	*NC
½	$> 10^4$	*< 20	< 20	< 20	< 20	< 20	< 20	$3{\cdot}16 \times 10^7$
1	< 20	$2{\cdot}34 \times 10^3$	*< 20	$1{\cdot}40 \times 10^2$	20	< 20	< 20	$> 10^4$
1½	< 20	< 20	$> 10^4$	*< 20	< 20	< 20	< 20	$> 10^4$
2	< 20	< 20	< 20	$> 10^4$	*20	< 20	< 20	$> 10^4$
2½	< 20	< 20	< 20	$3{\cdot}12 \times 10^3$	$> 10^4$	*< 20	< 20	$> 10^4$
3	< 20	< 20	< 20	$6{\cdot}40 \times 10^2$	$1{\cdot}66 \times 10^3$	$> 10^4$	*< 20	$> 10^4$
3½	< 20	< 20	< 20	$5{\cdot}40 \times 10^2$	< 20	$8{\cdot}00 \times 10^3$	$> 10^4$	$2{\cdot}40 \times 10^7$
5	< 20	< 20	< 20	< 20	< 20	< 20	< 20	$3{\cdot}16 \times 10^7$

*, Addition of nappy swatch.
NC, Not counted.
< 20, No colonies seen on plate.
$< 10^4$, Colonies too numerous to count at 2×10^{-1} dilution.

Table 2. *Total colony counts/ml in the serial urea insult test, using a formulation giving a fast available chlorine release*

Time (h)	1	2	3	4	5	6	7	8
0	*NC	NC	NC	NC	NC	NC	NC	*NC
½	$> 10^4$	*< 20	< 20	< 20	40	< 20	< 20	$2{\cdot}80 \times 10^2$
1	$> 10^4$	$> 10^4$	*< 20	< 20	< 20	< 20	< 20	$> 10^4$
1½	$2{\cdot}00 \times 10^3$	$2{\cdot}00 \times 10^3$	$> 10^4$	*< 20	< 20	< 20	< 20	$> 10^4$
2	< 20	< 20	$> 10^4$	$> 10^4$	*< 20	< 20	< 20	$> 10^4$
2½	$> 10^4$	< 20	20	$> 10^4$	$> 10^4$	*< 20	< 20	$> 10^4$
3	< 20	< 20	< 20	$4{\cdot}30 \times 10^3$	$> 10^4$	$> 10^4$	*< 20	$> 10^4$
3½	< 20	< 20	< 20	$1{\cdot}60 \times 10^2$	$> 10^4$	$> 10^4$	$> 10^4$	$2{\cdot}80 \times 10^7$
5	< 20	< 20	< 20	< 20	$2{\cdot}40 \times 10^2$	10^4	10^4	$2{\cdot}80 \times 10^7$

For notes, see Table 1.

Table 2 is representative of the pattern of results obtained with a fast chlorine release mixture showing adequate reduction when the urea content is low but totally unacceptable counts after addition of high amounts of urea. The product tested in this instance was based on sodium dichloroisocyanurate which is rapidly hydrolysed. In a carefully monitored home trial (not published) this formulation failed after the addition of four wet and only one soiled napkin. Two hours after the last addition, the soak water contained $1{\cdot}20 \times 10^5$ organisms/ml.

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DISCUSSION

Burn *et al.* (1969) considered less than 10000 organisms/ml of soak solution to be a satisfactory count in in-use conditions. We consider a product to pass the Serial Urea Insult Test if the number of bacterial surviving in the soak water is less than 100 organisms/ml 2 h after the last addition and less than 1000 organisms/ml between 1 h after the bacterial insult and the end of the test.

It may be considered too rigorous a test, but it has proved itself to be very satisfactory for assaying development products, allowing for large safety margins in recommended soak times, and also as a method for comparing commercially available nappy treatments.

However, it is important that whatever conclusions may be drawn from results of this laboratory test, they are confirmed by in-home surveys which are a necessary way of testing products with such practical implications.

This test has been evaluated by the Australian Government Authorities and has been adopted as the test for Assessment of Napkin Storage Products.

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