

Wheat and Milk Intolerance in Children With and Without Autistic Spectrum Disorder as Detected by Diallertest® Patches

A major project submitted in partial fulfilment for the award of the degree
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University of Wollongong

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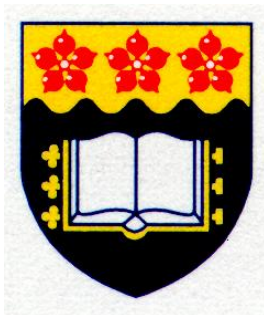


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Acknowledgements

The completion of this research project could not have been possible without the help many people. In particular I would like to express a special thankyou to the following:

Dr Velencia Soutter, Dr Anne Swain and Dr Rob Loblay, for their passion and knowledge in this area, their guidance and allowing me to gain invaluable experience about working and researching within a the setting of a clinical dietician. Thank you for allowing me the privilege of being able to work with such a dedicated and knowledgeable group of people.

Tim and Jason (IT RPAH) for their assistance in anything required for computers.

Karen Walton and Kelly Lambert (Lecturers UOW) for their patience throughout the project answering many questions and guidance in their lectures.

The dieticians and staff of the Allergy unit for their help and kindness, for making me feel so welcome and allowing me to use the lunchroom as my “office”.

Page Thody (Co-researcher) for all the telephone calls and support during this research process.

All the parents and children who participated in the study, without whom there would be no research to report on.

Last but not least, to all others, who have provided direct or indirect assistance to the project, and attempted to limit my stresses are also gratefully acknowledged. The preparation of this report would not have been possible without the support and valuable contributions of these individuals.

ABSTRACT

Background: Parents of children with Autistic Spectrum Disorder (ASD) often report that wheat and milk promotes undesirable symptoms and behaviours characteristic of food intolerance. Elimination-Oral-Food Challenges are the gold-standard diagnostic tool, but are difficult to complete. Diallertest® patches may be an easier alternative to predict delayed-reactivity (T-cell mediated) food intolerance. This study evaluated the usefulness of Diallertest® patches in predicting delayed-reactivity food intolerance in children with and without ASD in a clinical setting. Additional aims were to identify relationships between food intolerance, behaviour and learning difficulties, and to assess value of digital photographs as a method of diagnosing reactions.

Methods: One-hundred-and-twenty-three (51 ASD and 72 non-ASD) children (1-13 years) with suspected food intolerance and negative skin prick tests were recruited. Diallertest® patches (wheat, milk and control) were attached to the upper back and removed after 48h. Digital photographs recording reactions at 48h and 72h were emailed. Parents returned completed questionnaires regarding their child's behaviour, physical symptoms, dietary modifications and oral food challenges to the Allergy Unit for analysis. Data were analysed by SPSS 15.0 to calculate descriptive statistics and found to be not-normally distributed so non-parametric tests were used. Chi-square test and Fishers Exact test was used for testing specificity and sensitivity of Diallertest® from oral-challenges using a significance level of $p < 0.05$.

Results: Parents had modified diet for 47 (38%) children, 30 (81%) of which were milk-free and 25 (53%) on wheat-free diets. Thirty-one participants (25%) with Diallertest® patches had been oral-food challenged for milk and wheat. Diallertest® was found to have an overall agreement of 52% (14/27) for wheat and 50% (13/26) for milk reactions of all participants.

Conclusion: These results suggest that Diallertest® patches do not strongly predict food intolerance in this population. Digital photographs are useful in identifying reactions, but careful instruction is necessary to ensure clear and accurate records are taken. Further research with controlled oral-food challenges is required focusing on challenge reaction time and dietary effects, to more accurately determine usefulness of the test.

Wheat and Milk intolerance in children with and without Autistic Spectrum Disorder as detected by Diallertest® Patches

INTRODUCTION

Autism and Asperger's Syndrome are two developmental disorders that are classified under the umbrella of Autistic Spectrum Disorder (ASD). ASD is diagnosed by presence of symptoms like impaired social interaction skills, communication skills and a lack of symbolic or imaginative play (American Psychiatric Association 2000). Prevalence of ASD has increased from as little as 3 per 10000 (APA 2000) to conservative estimates of 13 per 1000. However, these values are considered to underestimate actual prevalence rates due to some children not being detected (Fombonne 2005). Increases in prevalence may be a result to changes in diagnostic criteria in the classification system and increased access to available services (Fombonne 2005, Harrington et al 2006). There is no definite cause or cure for these disorders, though dietary modification has been found in certain circumstances to improve behaviour, learning and social skills and autistic behaviours (Whiteley 1999).

The gluten- and casein-free (GFCF) diet is one such diet that has gained strong parental interest and support, despite limited definitive evidence to support its effectiveness (Levy et al 2003, Wong & Smith 2006, Hanson et al 2007). Parents of children with Autistic Spectrum Disorder (ASD) often report that the presence of milk and/or wheat in the diet can promote adverse behavioural problems, skin problems and gastrointestinal symptoms including hyperactivity, aggravation, language deterioration, vomiting and diarrhoea (Jyonouchi et al 2005, Kalach 2005, Whiteley 1999). These clinical features are not suggestive of IgE mediated allergies, (a conclusion confirmed by negative skin prick tests) but instead indicate that delayed-onset (T-cell mediated) intolerance to milk and wheat is a plausible mechanism. Scientific research has shown some evidence for such a link between ASD and food intolerance, but lacks quality blinded controlled studies.

Elimination diets and systemic oral challenges are the clinical gold-standard tool to diagnose food intolerance, and have demonstrated that milk and wheat protein intolerances can co-exist with intolerances to natural food chemicals and food additives present in food. Food chemical intolerances are considered as non-immunological

reactions (T-cell mediated), but the mechanism of reactions to milk and wheat proteins in this population are still yet to be determined. Oral-food challenges are complicated to conduct and parents of ASD children are often reluctant to complete oral-food challenges for fear of regression and backward-steps in learning and behaviour. These factors limit what statistically significant research can be conducted upon the effects diet plays upon behaviour and learning in populations with ASD.

Diallertest® is a new type of allergy skin test designed for topical application ('patch test'). Standard allergy tests ('skin prick tests') measure immediate (IgE mediated) immune reactions to food and other allergens, while the Diallertest® is intended to measure delayed (T-cell mediated) reactions occurring 24-72 hours after exposure. The test provides dry intact protein molecules to the skin surface to be solubilized through sweat secretion. The amount of protein deposited on the patch is constant, easily measurable with the total amount of protein released in a sustained fashion to the skin (du Toit 2006, Kalach 2005). Previous studies have found Diallertest® to be statistically significant for use in infants and children with atopic dermatitis (Heine et al 2006, Kalach et al 2005, Roehr et al 2000). As atopic dermatitis can be a symptom of food intolerance, it is expected that Diallertest® predictive values would be similar in food intolerant children (Niggermann 2000, Roehr 2000). Little is known about the value of patch tests like the Diallertest® in determining food intolerances for children with and without ASD. If valid, the Diallertest® could separate specific food-protein intolerances (eg wheat and milk) from more generalised food chemical intolerances and be a useful clinical tool to use alongside oral-food challenges for this population.

This study aimed to evaluate the usefulness of the Diallertest® patch test in diagnosing delayed (T-cell mediated) milk and wheat protein intolerances in children. The results were correlated with altered behavioural changes, clinical history and oral food challenge results to determine how predictive this test is for food intolerance in children with and without ASD. Secondary objectives were to observe possible mechanisms that may link milk and wheat intolerances to children with and without ASD, and to determine the suitability of digital photography in measuring and recording test results.

METHODS

Subjects

Participants with ASD were predominantly recruited from a cohort of 220 children who had been seen previously at RPAH Allergy Unit and recruited in Protocol X03-0197. Originally, 123 children (51 ASD and 72 non-ASD aged 1-13 years) with suspected food intolerance were recruited. Children exhibited at least one symptom of allergy, including digestive malfunctions (eg loose stools, colic, vomiting, gastroesophageal reflux and failure to thrive) and behavioural effects (eg hyperactivity, language and altered sleep patterns). Maximum age was 13 as parents are more likely to be in control of their child's diet and maintain an awareness of eating habits. Children above this age were excluded. Anti-histamine and corticosteroid treatments were discontinued at least 2 weeks before testing. Children were also excluded if they had documented evidence of immediate hypersensitivity or IgE-mediated reactions (positive skin prick tests) to milk or wheat to exclude the possibility of co-existing IgE-mediated food allergy. A sample size of 50 was chosen using a power calculation from a population of 220, with a 20% response rate, 90% confidence interval and 10% error margin. Additional children were recruited above this recommended sample size to account for incomplete data and dropouts.

Methods

This was a follow up study to a research project initiated in 2003 by the Royal Prince Alfred Hospital (RPAH) Allergy Clinic. Parents of previous ASD participants were telephoned and invited to participate. Parent Information Sheets were provided (Appendix 1) and children booked in. Non-ASD participants were recruited from new and follow-up patients routinely attending the Allergy Unit to make a control group. Upon appointment Parental Consent (Appendix 2) was signed, a diet history completed (Appendix 3) and a booklet of questionnaires with a Reply Paid envelope was provided (Appendix 4-8). Once completed questionnaires were returned to RPAH Allergy Clinic for analysis via postal service. Three Diallertest® patches (Diallertest, DBV-Technologies, Boulogne-Billancourt, France) for milk, wheat and control with no allergen were applied to the upper back.

Patches were removed after 48 hours, examined and read at 48 and 72 hours. Digital photographs recorded both of these readings and were emailed or posted to the Allergy

Unit for analysis. Reactions were considered positive if at 72 hours the tested skin exhibited a stronger skin reaction than the control due to the presence of clear redness and palpable infiltration or eczema as described by Majamaa et al (1999). A negative reaction had neither visible nor palpable changes on the skin and tended to get stronger by 72hrs. Irritation reactions included redness with no infiltration and tended to get weaker by 72hrs. A reaction to all patches including the control was regarded as hypersensitive and invalid for use. No itch, pain or discomfort was expected to occur.

Tools

Previous investigations in this broad study had developed and used a validated booklet of questionnaires designed for parents to complete about their child's diet, behaviour, eating patterns and oral-challenges. Many of these questionnaires were returned incomplete or unused with complaints about the amount of time required to complete them, inconvenience and inappropriateness for parents of ASD children. Our questionnaire was thus modified to reduce the amount of questionnaires that parents were required to complete (Appendix 4-8).

Data Analysis

All statistical tests were performed using SPSS 15.0. Data were tested for normality using Shapiro-Wilk test. All variables were found to be <0.05 thus not normally distributed so parametric testing was used including Chi-Square Test, Fishers Exact Test, Kappa's Measure of Agreement Test and Wilcoxon Signed Rank Test. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), overall agreement and p-values are presented for relationship between Diallertest® reactions and other variables. Where, sensitivity = number positive challenge identified as correct by Diallertest® (s) / total number of positive challenge; Specificity = number negative challenge identified as correct by Diallertest® (s) / total number of negative challenge; Positive predictive value (PPV) = number of positive challenges identified as correct by Diallertest® (s) / total number of positive Diallertest® results; Negative predictive value (NPV) = number of negative challenges identified as correct by Diallertest® (s) / total number of negative Diallertest® results; and Overall agreement = total number of children correctly classified by the Diallertest® / total number of children tested.

Financial Support

Nutricia Australia Pty Ltd provided Diallertest® devices free of charge. No financial support was provided. Participants were volunteers and received no payment or incentives to participate.

Ethics Approval

The Study was approved by the Ethics Review Committee (RPAH Zone) of the Sydney South West Area Health Service and written parental consent was obtained in all cases from parents. Participants were identified by code numbers to ensure anonymity with results entered into the password secured database at RPAH Allergy Clinic.

RESULTS

SPSS 15.0 was used to analyse data. The following results involved the comparison of a group of children with autism, ASD group (n=51) and without autism (n=72) against variables.

Abbreviations Used

ASD – Autistic Spectrum Disorder

RPAH – Royal Prince Alfred Hospital

PPV – Positive Predictive Value

NPV – Negative Predictive Value

SPT – Skin Prick Test

Table 1. Sample population characteristics

Characteristic	Non-ASD (n=62)	ASD (n=42)	All (n=104)
Sex^a			
Female	23	5	28
Male	39	37	76
Height^b (cm)	112	125	118.5
Weight^b (kg)	22	26	24
Age^b (years)	5	8	6.5

^aTotal gender number

^bMean

Table 1 shows characteristics of the two population groups within the study who returned the questionnaire, Diallertest® photograph results, or both. Some data were missing in the Non-ASD group, which may account for differences between the two groups

Table 2. Subject participation rates and characteristics.

N=123		ASD	Non-ASD	All Participants
Questionnaire	Distributed (n)	51	72	123
	Received (n)	39	37	76
	Response Rate	76%	51%	62%
Diallertest®	Distributed (n)	51	72	123
	Received (n)	37	59	97
	Response Rate	73%	82%	79%
Gender	Male (n)	43	47	90
	Female (n)	8	25	33
Age (mean years)		8	5	6

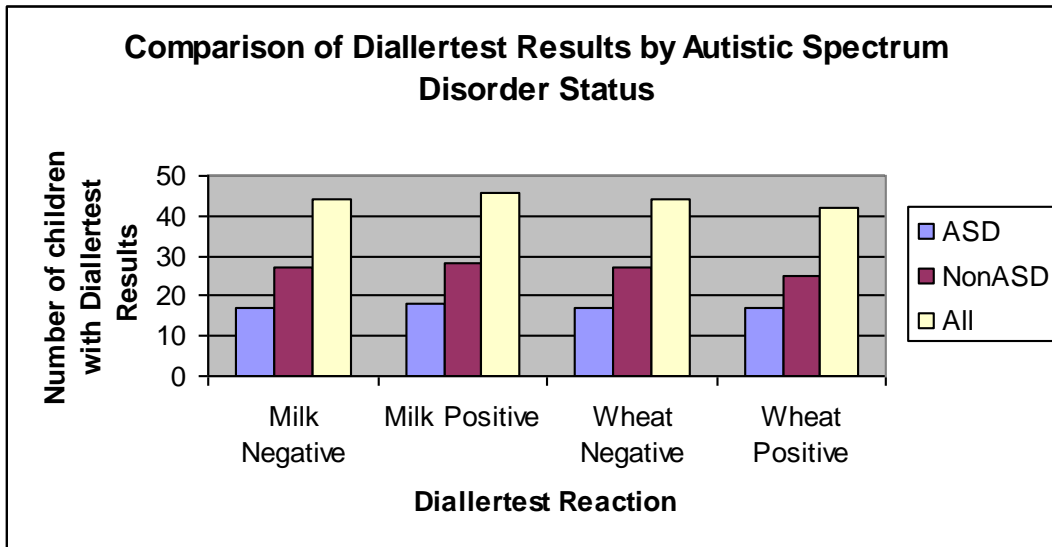
Table 2 summarises the number of questionnaire and Diallertest photographs that were distributed and returned. High response rates were observed. Of those who returned Diallertest reactions, 26 had completed milk oral-food challenges and 27 had completed wheat oral-food challenges (Table 3).

Table 3. Number and ASD status of children who returned Diallertest® Results and completed Oral-Food challenges for milk and/or wheat

	Milk Challenge	Wheat challenge
All (n)	26	27
ASD (n)	14	15
Non-ASD (n)	12	12

Children with ASD have a 49% chance of a milk reaction being positive and a 50% chance of a wheat reaction being either positive or negative. Children without ASD had a 51% chance of a milk reaction being positive and a 48% chance of a wheat reaction being either positive or negative (Figure 1).

Figure 1. Milk and wheat Diallertest® reaction according to ASD status



In calculating values for sensitivity, specificity and likelihood ratios of a positive and a negative test result for Diallertest®, we considered results of challenges, ASD, age, behaviour to determine any effect or significance they might have. Chi-square tests were used to find these values, with Fisher’s exact test being used instead of the chi-square p-value due to the small sample population, as values in the contingency table often being less than 6. A high male to female ratio was present in the ASD group compared to the non-ASD group and were checked for significant differences, but none were found. As evident in Table 4 predictive values for the Diallertest® were quite low for ASD, Non-ASD and all participants with non-significant p-values.

Table 4. Sensitivity, specificity, positive & negative predictive values for Diallertest® reaction in relation to clinical response to oral milk & wheat challenge

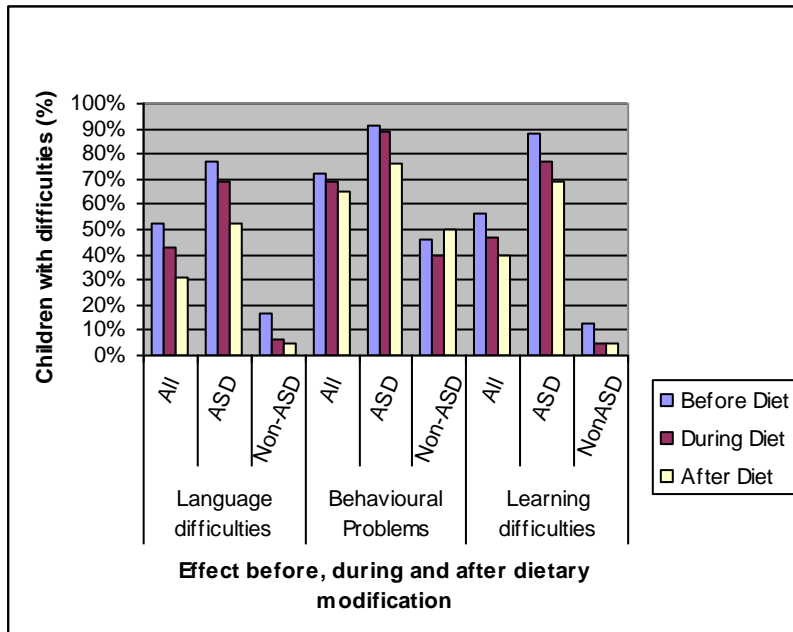
	Diallertest® Population Milk			Diallertest® Population Wheat		
	All n = 26	ASD n =14	NonASD n = 12	All n = 27	ASD n = 15	NonASD n = 12
Sensitivity (%)	55	67	45	50	57	45
Specificity (%)	33	40	0	56	50	100
PPV (%)	73	67	83	69	50	100
NPV (%)	18	40	0	36	57	14
Overall	50	57	42	52	53	50
Agreement (%)						
P value^a	0.491 (NS)	0.622 (NS)	0.500 (NS)	0.555 (NS)	0.595 (NS)	0.583 (NS)

^a P-value <0.05 significant

Kappa measure of agreement test was used to measure consistency between the gold standard elimination-oral-food-challenge and Diallertest® results. The Kappa Test found a measure of agreement value of –0.090 and 0.049 with a significance of 0.612 and 0.785 for milk and wheat respectively, indicating very poor agreement between the Diallertest reactions and Challenge results of both milk and wheat.

All other variables tested against the Diallertest®, were found to be non-significant. Gender male p 0.684 and 0.611, female p 0.500 and 0.750 were non-significant for both milk and wheat respectively. Age groups were non-significant with p-values of 0.333 for ages 4-6 and 0.500 for ages 7-9. Of all the children involved in the study, 39% (30/76) were found to be on milk free diets and 33% (25/76) did not consume wheat in their diet. Wilcoxon signed rank test was used to detect changes in behavioural problems, learning difficulties and language difficulties as a result of modifying diet to milk and/or wheat free diets. Statistically significant reductions were found in behavioural problems $z = 2.00$, $p < 0.05$ with a small effect size ($r = 0.20$), learning difficulties $z = 3.51$, $p < 0.005$ with a large effect size ($r = 0.53$) and language problems $z = 3.29$, $p < 0.05$ with a large effect size ($r = 0.49$) according to changes between before and after diet. Figure 2 demonstrates the changes of language, behaviour and learning difficulties for all participants during dietary modification.

Figure 2. Effect of Dietary modification upon language, behaviour and learning difficulties for children with and without ASD.



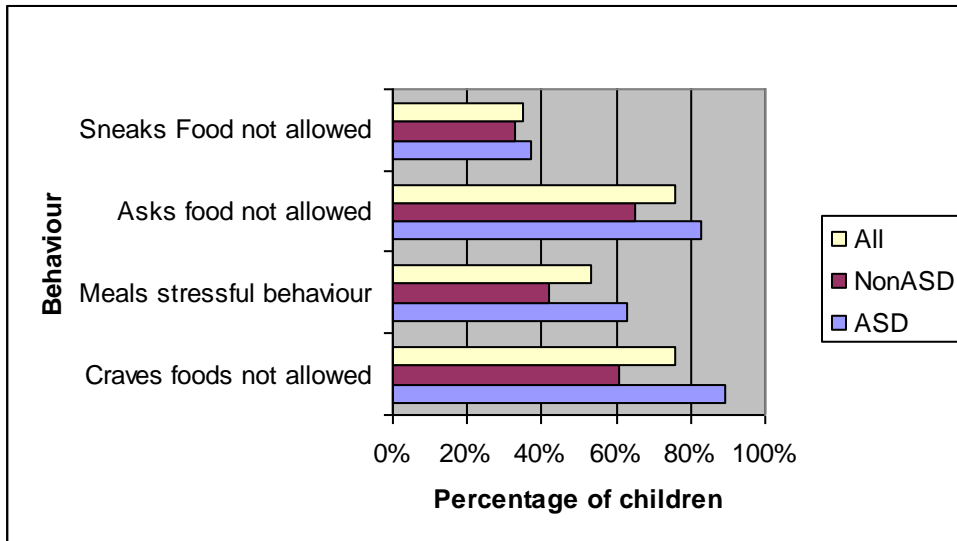
Diallertest®-Challenge results were additionally compared to the presence or absence of milk and wheat in the diet (Table 5) to determine if there was any relationship to the Diallertest® reaction observed. However, all values were found to be non-significant $p > 0.05$.

Table 5. Effect of milk or wheat-free diet upon predictive values of Diallertest® against oral-food challenges

	Challenge-Diallertest® Milk		Challenge-Diallertest® Wheat	
	No Diet	Milk Free	No Diet	Wheat Free
	n = 11	n = 15	n = 11	n = 16
Sensitivity (%)	60%	53%	17%	67%
Specificity (%)	33%	0%	40%	75%
PPV (%)	43%	100%	25%	89%
NPV (%)	50%	0%	29%	43%
Overall	45%	53%	27%	69%
Agreement (%)				
P value^a	0.652	(NS)	0.197	0.192
	(NS)		(NS)	(NS)

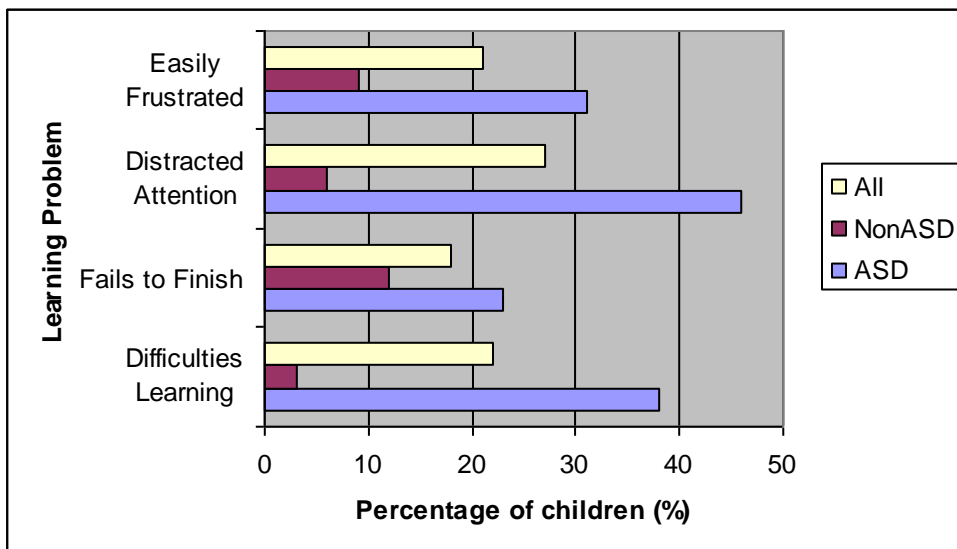
^a P-value <0.05 significant

Figure 3. Parent-reported eating behaviour of children with and without Autistic Spectrum Disorder



Conner's Rating Scale results were separated into specific categories and analysed for differences between ASD and non-ASD groups (Figure 4).

Figure 4. Parent-rated 'Very Much' scores on Conner's' Learning Scale for Learning Problems



DISCUSSION

The main question raised in this study was if the Diallerstest® was useful diagnostic tool in predicting specific food intolerances in children with and without ASD. To our knowledge, this is the first study where Diallerstest® has been tested on ASD children. This study found its male:female ratio of autistic children to be comparative with the mean male:female ratio of 4:1 found by Fombonne 2005. Boys thus appear to be more susceptible to developing autistic spectrum disorders.

Study procedure was standardized with all reactions photographed and filed in a central computer to allow posterior controlled readings of the investigator to be made. As diagnosis of Diallerstest® reactions are qualitative, subject to human errors in performance and interpretation, the result was confirmed by all investigators to overcome this factor.

Our study did not skin prick test (SPT) or measure IgE levels assuming that they measured IgE immune reactions while Diallerstest® measured non-immune delayed-reactivity (T-cell mediated). All subjects had previously conducted negative SPT, which indicates the absence of IgE mediated allergic reactivity but fails to detect delayed-onset non-IgE mediated allergies (De Bouissieu 2003, du Toit 2006, Kalach 2005). Around 10% of positive DBPCFC results are not IgE mediated. Because of this, food-related symptoms are better in indicating the need for food challenges to diagnose allergy (Niggemann 2001). This fits with the findings of Majamaa et al (1999) where milk patch tests were positive in 44% of positive milk challenge patients, the majority with negative SPT results. Milk challenge-positive participants included 14% who had positive SPT (2 being delayed reactions to challenge) and 26% with elevated IgE levels (10 with delayed-reactions to challenge). Positive patch tests were highly associated with delayed-type reactions (Isolauri 1996). Skin patch tests were found to have a PPV of 43% for positive milk challenge patients (Majamaa 1999).

Studies show inconsistent results on patch test reliability for predicting food intolerance in various populations (Roehr 2001). Significance was obtained in populations of children and infants with atopic dermatitis, protein-induced enterocolitis syndrome and cow's milk allergy with digestive symptoms (de Boissieu 2003, Fogg 2006, Heine 2006).

Predictive values vary greatly, with specificities of 71% - 99%, sensitivities of 9% - 100%, PPV 75% - 95%, NPV 71% - 100% with overall test accuracies of 83% when compared to oral food challenge outcomes (De Boissieu 2003, du Toit 2006, Fogg 2006, Heine 2006, Kalach 2005, Niggemann 2000, Saarinen 2001). If patch tests were standardized it could improve diagnostic accuracy of this non-invasive tool (Kalach 2005).

As age decreases, number of false-negatives increase (de Bouissieu 2003, Kalach 2005). Conversely, Saarinen et al 2001 found patch testing more accurate in diagnosing immediate-reactions rather than delayed-reactions, which may be accredited to a population of newborn infants. According to Niggemann 2000, patch testing only works on people who have atopic dermatitis. This statement could be confirmed by our findings, with little overall agreement between Diallertest® and challenge results (Table 4). However, the absence of visible reactions may not mean the absence of pathology. Mechanisms are different for skin T-cells and gastrointestinal T-cells, making it difficult to rationalise a relationship between gastrointestinal reactions and skin reactions from a food trigger. Fogg et al. suggested that the T-cells may be migrating through the circulatory system or interacting in regional lymph nodes. A recent study by Girardi (2005) discovered that while skin reactions are negative, histological changes with lymphocyte proliferation still occurred following exposure to patch testing. This suggests that children with negative skin reactions to Diallertest® may have histological changes attributed to T-cell mediated reactivity.

Diallertest® was not found to be significant for any combination of variables with this population of ASD and non-ASD children. No differentiation was made between the traits and intellectual levels, scales and severity of ASD – all which may influence reaction outcome.

Effects of Milk and Wheat upon behaviour and learning

According to his parents, one child's autistic traits almost completely disappeared as a result of eliminating milk and wheat from his diet. It is uncommon for autistic symptoms to be completely ameliorated (Howlin 1997). As expected, behavioural problems were significantly greater in children with ASD than those without.

Photographs

Some parents were found to lack, or were unfamiliar with equipment and technology required to email digital photo results. The majority of photographs were clear, however there were some reduced quality photos that were impaired, blurry, out of range, with bad lighting and contrast. This complication could be easily overcome through clear instructions upon taking digital photographs and methods of attaching them to email.

Adverse Reactions

Like the standard SPT, Diallertest® allergen only enters the superficial layers of the skin and is not absorbed into the bloodstream. Therefore it does not stimulate the immune system as a whole and negative systemic effects were not expected. Previous studies have recorded no immediate reaction in first hours of testing, or side effects due to application of Diallertest® (Kalach 2005). Conversely, a few parents in our study reported that the application site remained with red, raised marks for over a week after the test was removed, with one child having itchiness develop at the reaction site a week after application. One extreme reaction was noted for a particular child who exhibited a rash over his body, fever and sleeplessness and irritable behaviour after Diallertest® application.

Study Limitations and Implications for Future Research

The methodological problems of this research must be considered when interpreting results. Parents, children or researchers were not blinded to the test and test results. Even though a large initial population was recruited and high response rates achieved (Table 2) few participants had previously completed challenges for milk and wheat, potentially impacting the strength of the study results. The study needs replication using larger and less heterogeneous samples, in regards to age, ASD severity, medical history and symptoms. Children with ASD vary greatly in terms of their behavioural and developmental traits and levels (Elder et al 2006).

Parental reporting and interpretation is known to have experimental flaws. While valuable for assessing behavioural changes, it cannot conclusively determine effectiveness of dietary modification. Clinical history and oral food challenge are most reliable for diagnosing non-IgE mediated food-induced allergic reactions, with challenges being the gold standard (du Toit 2006), however there is no universally accepted standard

for its use (Majamaa et al 1999). Clinical double blind challenges would provide more conclusive results and distinguish between acute and delayed-onset reactions to increase validity of Diallertest®. However, as noted by Whiteley et al 1999, if exposing children with ASD to suspect food has adverse effects, there may be significant risks, ethical issues and parental objections to overcome (Fogg 2006). In a study conducted by Fogg (2006), 3/30 challenges required intravenous fluid therapy and prolonged monitoring following oral-food challenge.

We were limited to only 26 and 27 children who had both Diallertest® and oral-food challenges. Hurdles to conduct challenges include uncompliant young children and children being unable to reach the top challenge dose required (Du Toit 2005). Parents of ASD children responding positively to diet are often hesitant to challenge due to fears of their child regressing backwards with permanent bad effects. Children can grow out of allergies and intolerances, indicating that food sensitivity can diminish with age. There was no information provided about when challenges had been conducted and whether they were currently applicable to each child. Additional study limitations may be due to human error in manual data entry, analysis and initial patch application as some milk and control patches appeared to be mixed up. Not all questionnaires returned were completely filled in, resulting in incomplete datasets.

Applying the Diallertest® patches on children with ASD did prove difficult in many circumstances. Some autistic children were fearful of taking the test going into panic attacks. Other subjects scratched the patches off on multiple occasions and had to re-apply new patches. An additional hazard was found with parents letting children go swimming or jump into the bath and wet the patches, which needed to stay dry for the full 72hrs. These factors account for some of the dropouts.

While records were made regarding any dietary modification, connection between diet and severity of Diallertest® were not determined. There is currently little research upon the effect that presence or elimination of the suspected food has upon outcome. Double-blind studies testing this could greatly impact significance of this test to find a suitable population. The use of codes or symbols for Diallertest® patches instead of letters, with different investigators applying and coding to those recording reaction having no knowledge of child history would ensure double-blinding and prevent bias in diagnosis.

CONCLUSION

This study found Diallertest® patches to have low correlations with oral-food challenges for milk and wheat, thus being weak predictors of delayed-reactivity food intolerance for children with and without Autistic Spectrum Disorder. Oral-food challenges are still necessary for diagnosis of T-cell mediated food protein intolerance. Digital photographs are useful in identifying reactions, but careful instruction is necessary to ensure clear and accurate records are taken. Future research with controlled oral-food challenges should focus on challenge reaction time and the effect of dietary elimination of suspect foods upon subsequent Diallertest® reaction, to more accurately determine usefulness of the test.

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Appendix 1. Parent Information Sheet

Dr Velencia Soutter

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ROYAL PRINCE ALFRED HOSPITAL

A tradition of excellence since 1882

Evaluation of Diallertest[®] in food intolerant children with and without Autistic Spectrum Disorder (ASD).

Information for Parents / Guardians

You and your child are invited to participate in a study to evaluate a new skin test device used for the diagnosis of cows' milk or wheat protein intolerance. You are being approached either because you and your child have previously participated in a study of diet and nutrition in children with Autistic Spectrum Disorder (ASD), or because your child has symptoms suggestive of food intolerance.

The study is being conducted by Dr Velencia Soutter, Dr Anne Swain and Dr Robert Loblay at the Royal Prince Alfred Hospital (RPAH) Allergy Unit, together with 2 dietetic students (Ms Page Thody & Miss Gemma Sampson).

Diallertest[®] is a new type of allergy skin test designed for topical application ('patch test'). Whereas standard allergy tests ('skin prick tests') measure immediate immune reactions to food and other allergens, the Diallertest[®] is intended to measure delayed reactions occurring 24-72 hours after exposure. As with the standard skin prick test, the Diallertest[®] allergen only enters the superficial layers of the skin and is not absorbed into the bloodstream. Therefore it does not stimulate the immune system as a whole and no general adverse effects are expected.

The allergen is applied to the skin surface on the upper part of the back with a plastic applicator, and is covered with a non-irritant protective film ('patch'). The patch is removed at 48 hours and the site of application is examined 24 hours later to record any

reaction. A positive reaction consists of a 1-2 cm area of localized redness at the contact site. There should be no itch, pain or discomfort from the test.

Three patches will be applied to your child's back: one with milk protein, one with wheat protein and a 'control' patch with no allergen. You will be asked to remove the patches after 48 hours. When you return to the clinic the following day, the size of any positive reaction will be measured and a digital photograph will be taken of the area. If you live some distance away and are unable to return to the clinic, we will ask you record and measure any positive reaction and take a digital photograph of the area. The result and photograph can be sent to us by email.

If you agree to participate your child's results will be incorporated into the Allergy Unit medical record and database. All information obtained during this study will be treated confidentially. Only the investigators and authorized Allergy Unit staff will have access to your child's information.

A summary of the results may be published in medical journals or presented at conferences. However, you and your child will not be identified in any such publication or presentation.

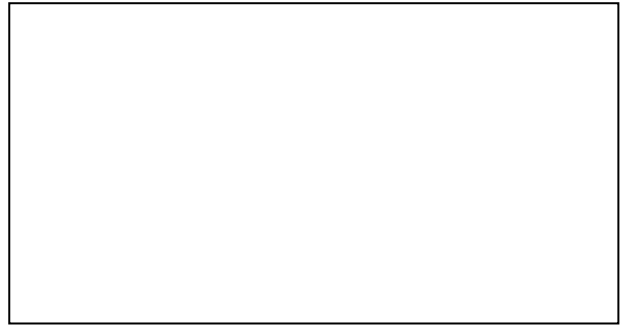
You are under no obligation whatsoever to participate. Whatever decision you make will not influence your medical care or relationship with Allergy Unit staff.

If you have any questions, or would like to discuss the study further, please contact Dr Velencia Soutter on 0417 217 174 or by email: velencia.soutter@email.cs.nsw.gov.au

This study has been approved by the Ethics Review Committee (RPAH Zone) of the Sydney South West Area Health Service. Any person with concerns or complaints about the conduct of a research study can contact the Secretary on 02 9515 6766 and quote protocol number X07-0000.

This information sheet is for you to keep.

Appendix 2. Parent Consent Sheet



ROYAL PRINCE ALFRED HOSPITAL
A tradition of excellence since 1882

**Evaluation of Diallertest® in food intolerant children with
and without Autistic Spectrum Disorder (ASD).**

PARENT / GUARDIAN CONSENT FORM

I, [name of parent/guardian]
of[address],

parent/guardian of [name of child]

have read and understood the Information for Parent/Guardian on the above research
study and have discussed the study with

I have been made aware of the procedures involved in the study, including any known or
expected inconvenience, risk, discomfort or potential side effect and of their implications as
far as they are currently known by the researchers. I also understand that the information
collected about my child will be included in the medical record and kept strictly confidential.

I freely choose to allow my child to participate in this study. I also understand that I can
change my mind without affecting his/her medical care or relationship with Allergy Unit staff.

I hereby agree to my child's participation this research study.

**NAME OF
PARENT/GUARDIAN:**.....

SIGNATURE:..... **DATE:**

NAME OF WITNESS:.....

SIGNATURE OF WITNESS:

Appendix 3. Diet History Sheet

Appendix 4. Follow-Up Questionnaire

Dietary Issues in children with and without Autistic Spectrum Disorder (ASD)

Follow-Up Questionnaire

ID: _____ **Date of Birth:** __ __ / __ __ / __ __ **Date:** _____

Please tick the boxes and answer the questions to the best of your recollection.

Put a question mark if you are uncertain or cannot remember.

1. When you first expressed an interest, was your child on a modified diet?

- NO YES

If yes, what modification had you made?

- | | |
|--------------------------------------|---|
| <input type="checkbox"/> Wheat free | <input type="checkbox"/> Sugar free |
| <input type="checkbox"/> Gluten free | <input type="checkbox"/> Artificial colour free |
| <input type="checkbox"/> Milk free | <input type="checkbox"/> Preservative free |
| <input type="checkbox"/> Dairy free | <input type="checkbox"/> Low salicylate |
| <input type="checkbox"/> Soy free | <input type="checkbox"/> Low amine |
| <input type="checkbox"/> Other | |

2. Did you see the dietitian as part of your involvement in the study?

- NO YES

If yes, what additional changes did you make?

- No extra changes

Reason _____

(e.g. too difficult, child refused, spontaneous improvement, happy with current diet, etc.,)

- | | |
|--------------------------------------|---|
| <input type="checkbox"/> Wheat free | <input type="checkbox"/> Sugar free |
| <input type="checkbox"/> Gluten free | <input type="checkbox"/> Artificial colour free |
| <input type="checkbox"/> Milk free | <input type="checkbox"/> Preservative free |
| <input type="checkbox"/> Dairy free | <input type="checkbox"/> Low salicylate |
| <input type="checkbox"/> Soy free | <input type="checkbox"/> Low amine |
| <input type="checkbox"/> Other..... | |

3. Did you notice any improvement?

- NO YES

If yes, how long did it take?

4. What symptoms did your child have?
 (a) Before coming to the clinic,
 (b) At his/her best on the Elimination diet, and
 (c) How are those symptoms now?

Indicate their severity at each stage by circling the relevant numbers:

SYMPTOMS		NONE	MILD	MOD	SEVERE
Behavioural problems	Before	0	1	2	3
	On diet	0	1	2	3
	Now	0	1	2	3
Language	Before	0	1	2	3
	On diet	0	1	2	3
	Now	0	1	2	3
Learning difficulties	Before	0	1	2	3
	On diet	0	1	2	3
	Now	0	1	2	3
Sleep disturbances	Before	0	1	2	3
	On diet	0	1	2	3
	Now	0	1	2	3
Reflux, vomiting	Before	0	1	2	3
	On diet	0	1	2	3
	Now	0	1	2	3
Wind, tummy pain	Before	0	1	2	3
	On diet	0	1	2	3
	Now	0	1	2	3
Loose stools, diarrhoea	Before	0	1	2	3
	On diet	0	1	2	3
	Now	0	1	2	3
Headaches	Before	0	1	2	3
	On diet	0	1	2	3
	Now	0	1	2	3
Mouth ulcers	Before	0	1	2	3
	On diet	0	1	2	3
	Now	0	1	2	3
Blocked/runny nose, etc.,	Before	0	1	2	3
	On diet	0	1	2	3
	Now	0	1	2	3
Ear infections, glue ears	Before	0	1	2	3
	On diet	0	1	2	3
	Now	0	1	2	3
Limb aches & pains	Before	0	1	2	3
	On diet	0	1	2	3
	Now	0	1	2	3
Skin rashes	Before	0	1	2	3
	On diet	0	1	2	3
	Now	0	1	2	3
Other (Please specify)	Before	0	1	2	3
	On diet	0	1	2	3
	Now	0	1	2	3

5. Did you do any challenges?

- NO YES

If yes, please list the reactions your child had to challenges:

- Describe the main symptoms you recall after each challenge. If you're not sure, or can't remember, put a question mark (?). For those challenges not done, write "N/D".
- Indicate the severity of reactions by circling the relevant numbers.

CHALLENGE	Type	SYMPTOMS	None	Mild	Mod.	Severe
Milk / dairy	Food		0	1	2	3
Wheat	Food		0	1	2	3
Colourings	Capsule		0	1	2	3
(102-155)	Food		0	1	2	3
Preservatives *	Capsule		0	1	2	3
	Food		0	1	2	3
Antioxidants	Capsule		0	1	2	3
(310-321)	Food		0	1	2	3
Nitrates	Capsule		0	1	2	3
(249-252)	Food		0	1	2	3
Propionates	Capsule		0	1	2	3
(280-283)	Food		0	1	2	3
Salicylates	Capsule		0	1	2	3
	Food		0	1	2	3
Amines	Capsule		0	1	2	3
	Food		0	1	2	3
MSG	Capsule		0	1	2	3
(621)	Food		0	1	2	3

* benzoates (210-213), sorbates (200-203), sulphites (220-228)

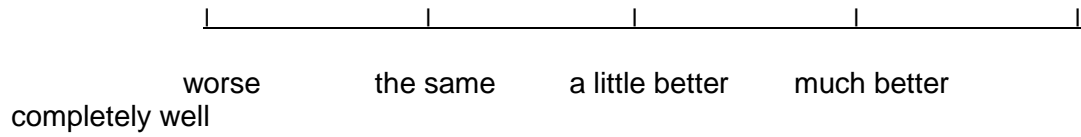
6. Do you still have your child on a modified diet at present?

- NO YES

If yes, what modification is your child on at present?

- | | |
|--------------------------------------|---|
| <input type="checkbox"/> Wheat free | <input type="checkbox"/> Sugar free |
| <input type="checkbox"/> Gluten free | <input type="checkbox"/> Artificial colour free |
| <input type="checkbox"/> Milk free | <input type="checkbox"/> Preservative free |
| <input type="checkbox"/> Dairy free | <input type="checkbox"/> Low salicylate |
| <input type="checkbox"/> Soy free | <input type="checkbox"/> Low amine |
| <input type="checkbox"/> Other..... | |

7. Overall, how is your child now compared with when you first brought him/her to the Clinic? *(please circle)*



Any Comments?

.....

.....

.....

.....

.....

.....

.....

.....

Thank you for taking the time to answer this questionnaire.

Appendix 5. Connor's Rating Scale

Appendix 6. Children's Eating Behaviour and Appetite Scale

CHILDREN'S EATING BEHAVIOUR & APPETITE SCALE

Child's Initials: ___ ___ : ___ ___ **Date of birth:** ___/___/___ **I.D.:** _____ **Date:** _____
(first name : family name)

Please read each statement and circle a number to indicate how much the statement applies your child *in the past six months*.

Not at all	Just a little	Pretty much	Very much	CEBAS - 50
0	1	2	3	1. Has poor appetite
0	1	2	3	2. Diet lacks good nutrition
0	1	2	3	3. Eats different foods from other children in the family
0	1	2	3	4. Eats different foods from main carer (e.g. mother)
0	1	2	3	5. Has fussy eating behaviour
0	1	2	3	6. Eats only small meals
0	1	2	3	7. Eats unusually large amounts of food
0	1	2	3	8. Lacks variety in food choices
0	1	2	3	9. Prefers bland foods
0	1	2	3	10. Makes repetitive food choices
0	1	2	3	11. Craves some types of foods
0	1	2	3	12. Is obsessed with sameness of foods
0	1	2	3	13. Is fussy about food temperature
0	1	2	3	14. Will only accept food or drink with certain plates or cups
0	1	2	3	15. Is fussy about food texture
0	1	2	3	16. Is fussy about food colour
0	1	2	3	17. Is afraid of trying new foods
0	1	2	3	18. Holds mouth closed to avoid eating food
0	1	2	3	19. Spits out foods after putting it in mouth
0	1	2	3	20. Lets food sit in mouth
0	1	2	3	21. Lets food fall out of mouth to reject it
0	1	2	3	22. Pulls food out of mouth after taking a mouthful
0	1	2	3	23. Refuses food by batting spoon away or pushing plate
0	1	2	3	24. Has trouble chewing food as expected for age
0	1	2	3	25. Vomits during or straight after eating
0	1	2	3	26. Gags on foods during meals
0	1	2	3	27. Has problems with choking at mealtime

Not at all	Just a little	Pretty much	Very much	CEBAS - 50
0	1	2	3	28. Spits/gags or refuses chunks in food or lumps of meat
0	1	2	3	29. Feeds self as expected for age
0	1	2	3	30. Won't eat without being spoon-fed
0	1	2	3	31. TV needs to be on to distract child to eat
0	1	2	3	32. Has trouble staying at the table to eat — up & down between mouthfuls
0	1	2	3	33. Is allowed to choose foods at mealtimes from what is served
0	1	2	3	34. Takes more than half an hour to eat meals
0	1	2	3	35. Asks for food between meals
0	1	2	3	36. Won't eat at mealtimes, but snacks between meals
0	1	2	3	37. Hides food to avoid eating it
0	1	2	3	38. Complains about being fat, and this affects food choices
0	1	2	3	39. Complains about being hungry between meals
0	1	2	3	40. Makes mealtimes stressful because of behaviour
0	1	2	3	41. Food reactions or special diet make it difficult to find suitable foods for child
0	1	2	3	42. Special diet is a strain on family food budget
0	1	2	3	43. Hard to prepare suitable meals for child
0	1	2	3	44. Special diet interferes with family lifestyle expectations
0	1	2	3	45. Special diet causes social isolation
0	1	2	3	46. Relatives complain about child's food issues
0	1	2	3	47. Relatives don't support child's special dietary your child
				If your child is on a special diet
0	1	2	3	48. Asks for food that is not on the "ALLOWED" list
0	1	2	3	49. Sneaks food that is on the "AVOID" list
0	1	2	3	50. Grabs or sneaks food that is on the "AVOID" list when away from home

<i>List your child's FAVOURITE foods</i>	<i>List your child's MOST DISLIKED foods</i>
1.	1.
2.	2.
3.	3.
4.	4.
5.	5.

Appendix 7. Diallertest® Photograph Results



Application of Diallertest Patches – Milk (M) Wheat (W) Control (C)



Positive Wheat Diallertest® Result 72hr photo



Diallertest® Reaction to Milk (M) and Wheat (W) 72hr photo



Diallertest® reaction to Milk (M) Wheat (W) and Control (C) 72hr photo