

Towards understanding the predictive capacity of Diallertest Wheat and Milk patches in children with and without Autistic Spectrum Disorder.

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of the degree of

Master of Nutrition and Dietetics

by

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Abstract

Introduction: Wheat and milk sensitivities are common in children and those with Autistic Spectrum Disorder (ASD) may be more susceptible than others. (Horvath K M.D. and Perman, J M.D.,2002, Wakefield AJ et al, 2002).Traditionally elimination diets followed by oral challenges are conducted to determine food intolerance. The Diallyrtest Atopy Patch Test is designed to register T-cell mediated late phase food sensitivity and is proposed as a alternate diagnostic measure.

Aim: To evaluate the validity of Diallyrtest wheat and milk patches as a determinant of wheat and milk intolerance in children.

Method: Diallyrtest wheat and milk atopy patches were tested on children aged one to thirteen years (N=123). Reactivity to APT was measured against milk and wheat oral challenge results previously conducted in a subgroup of the same children (N=31). Sensitivity, specificity, positive predictive value, negative predictive value and overall agreement of the patches were determined by statistical analysis.

Results: While some of our results showed similar trends to those of previous studies (Niggemann et al, 1999, Canani et al, 2007, Fogg et al, 2006) the predictive value as demonstrated by overall agreement was poor.

Conclusion: Notwithstanding its success in the literature, preliminary findings of this study suggest a limited predictive value for Diallyrtest wheat and milk patches in regard to wheat or milk reactivity from oral challenge. However, a limitation in this particular study is the interlude between the oral challenge and the patch testing, as changes in reactivity may have occurred during this time.

Although our study was flawed by factors relating to the comparative measure it seems evident that Diallyrtest patches measured heightened reactivity in over 50 % of participants. Although we cannot clarify the predictive capacity of Diallyrtest at present neither can we dismiss the potential value of this product.

Introduction:

Autistic spectrum disorder (ASD) is a developmental cognitive disorder clinically characterised by impaired social interaction and communication and restricted and often repetitive behaviours. (Fernell Elizabeth et al, 2007). The precise aetiology of ASD is unknown, numerous genes have been implicated and irregularities at almost every chromosome have been observed. (Castermans, D 2004). ASD is four times more common in boys than girls, therefore, an X linkage is suspected. (Jamain, et al 2003., Trottier, G, et al 1999). Epilepsy is a pathology apparent in ASD and it is believed that 46 % of all cases experience seizures. (Hughes JR, 2007)

Food selectivity is a well known behavioural characteristic of people with ASD, whether this stems from either oral and odour hypersensitivity or hyposensitivity (Bennetto L, 2007) or is an adaptive behaviour stemming from gastrointestinal sensitivity is not known. Alternatively this characteristic may simply be an ASD behavioural tendency relating to desire for sameness and ritual, therefore ASD food selectivity is in itself a typical example of the heterogeneity of this condition.

Gastro-intestinal sensitivity is a common finding in persons with ASD as well as their immediate relatives. (Horvath K M.D. and Perman, J M.D., 2002, Wakefield AD et al,) Permeability of the intestine permits “exorphins” which are exogenous opioids derived from wheat and milk to pass through the intestinal wall into the blood stream and through the blood-brain barrier where they disrupt normal neurological function. It is believed that these metabolites from wheat and milk and potentially other chemicals from food as well, may cause inflammation and trigger an immune cascade response. It has further been suggested that this “immune response” may result in an autoimmune reaction similar to that seen in arthritis or multiple sclerosis, whereby cells and tissues of the body or brain itself are attacked and destroyed. (Trottier et al, 1999, Weizman et al 1982., Singh et al, 1993., Burger RA and Warren RP, 1998.)

Elevated levels of cytokines have been found in peripheral blood mononuclear cells of ASD children in response to wheat and whole cows’ milk protein, particularly TNF (tumour necrosis factor) and Interleukin- 12 compared to controls. (Chez MG MD et al, 2007, Jyonouchi H MD et al, 2005) “Interleukin- 12 has been regarded by many immunologists as

the initiator in the early stages of the autoimmune mechanism; it could be the inducer, of early events that cause autism.” (Singh, V. K., 1996)

Research in the field of autism has revealed anatomical differences in the brains of persons with ASD. Aberrant cell-mediated and antibody responses to myelin basic protein have also been well documented. (Weizman et al 1982., Singh et al, 1993., Burger RA and Warren RP, 1998.)

Additionally, biochemical assays have outlined a range of disturbances which include amongst others: a high blood copper/zinc ratio (Holford, Patrick 2003), defects in the methylation pathway(Osiecki, Henry 2006), impaired serotonin synthesis and increased serum free tryptophan as well as elevated kynurenine (a by-product of tryptophan) (Murray, Pizzorno and Joiner-Bey 2002). Deficiencies of various micro-nutrients have also been observed (Magnesium and Vitamin B6 being well documented.) (Holford 2003).

Aside from autism spectrum disorder, many children in the general population are milk and wheat intolerant. This is usually detected soon after birth or around the age of weaning. Common symptoms in babies are reflux, retching, diarrhoea, colic, rashes and failure to thrive. Most of these babies will be trialled on soy formulas and if this is not an effective substitute then hydrolysed peptide formulas such as Neocate or Elecare will then be introduced.

When food intolerances are suspected, elimination diets are recommended to remove the offending substances. Evidently, it is extremely important to ensure that the child receives optimum nutrition from alternative foods. In practice, however, restricted diets have been known to contribute to nutritional deficiencies. (Arnold G et al, 2003).

Table 1. Types of food intolerance, immune mediation and related symptoms:

Types of food Intolerance	Types of food Intolerance	Types of food Intolerance	Types of food Intolerance
Allergy	Non-allergic food hypersensitivity	Non-allergic food hypersensitivity	
	Amines, salicylates,preservatives		
IgE mediated	Non-T-cell mediated	T-cell mediated	Mixed IgE and non-IgE reactions
Urticaria	Behavioural	Enterocolitis,	Atopic dermatitis
Facial oedema		Enteropathy	Oesophagitis,
Hayfever		Rectocolitis	Gastroenteritis,
Digestive anaphalaxis			Colitis
Anaphalactic shock			Infant colic

Table 2. Standard methods for detecting food intolerance:

Clinical methods used for detecting Types of food intolerance are:	Methods:
IgE mediated reactions	Skin prick tests or RAST (radioallergosorbent tests)
Non T-cell mediated reactions(salicylates, amines and preservatives).	Elimination diets and oral challenge
T-cell mediated reactions.	Patch tests, Peripheral blood mononuclear (PBM) cell antigen response, Elimination diet and oral challenge
ELISA (enzyme linked immunosorbent assays)	Food industry for allergen detection
Breath test	Lactase deficiency
Human leukocyte antigen (HLA) – typing	Coeliac disease
Biopsy	Coeliac disease

“Allelic products of certain HLA genes are associated with autism, including the null allele of the C4B gene. (Trottier et al, 1999. Burger R, 1998). A null allele is a deficient gene from which no product is produced. An inherited C4B null allele results in a deficiency of C4B protein (a subset of the C4 complement protein) and a breakdown in the classical complement pathway. In humans, normal plasma contains nine (C1-C9) heat labile proteins of the complement system. These proteins augment the antibacterial activity of antibodies through a cascade of interdependent reactions called the classical complement pathway. A haplotype is the particular combination of HLA alleles found of the various HLA genes on an individual chromosome, it was found that an extended haplotype of the DR4 allele is increased more

than sixfold in autism and three extended haplotypes of the DR7 allele occur about twice as frequently in individuals with autism than controls.” (Burger R et al, 1998).

“Although environmental factors have not been shown to be a cause of autism, they may well act as second ‘hits’ in genetically susceptible individuals.” (Castermans D et al 2004).

How might this happen?

Lymphocytes divide into B and T cells, there are two types of T-cells namely: CD8 or Cytotoxic (killer T-cells) which destroy virally infected cells and tumour cells and CD4 or Helper T-cells which activate B cells and macrophages. T and B cells have antigen binding receptors. Mature lymphocytes are receptor specific to only one particular antigen. For T-cells to become activated they must bind to a foreign antigen and an HLA (human leukocyte antigen) complexed to that antigen, (the HLA-complex is expressed on the surface of an antigen presenting cell such as a macrophage or B cell). (Burger R, 1998). When irregularities occur in HLA, T cells either cannot respond to antigens or may be over activated such as in autoimmune conditions. (Warren et al, 1995, Plioplys et al,1994,).

Aims:

- ▣ To evaluate the validity of Diallestat wheat and milk patches as a determinant of wheat and milk hypersensitivity in children with and without Autistic Spectrum Disorder.
- ▣ To evaluate the usefulness of digital photography as a means of measuring and recording atopic patch test (APT) results.

Methods:

Ethics approval was sought and obtained by Dr Robert Loblay, through extension of the previously held ethics approval in regard to “Dietary Issues in Children with and without ASD.”

ASD Participants (N=51) were recruited from the RPA Allergy Clinic ASD data base while non-ASD participants (N=72) were either siblings of the ASD children or were drawn from the daily clinical attendance at RPA Allergy Unit. Parents of the ASD group were contacted by telephone and their children were invited to participate in the study. The study entailed the

Diallertest APT, a questionnaire, a diet history and the proviso that parents record the results photographically for submission to the RPA clinic.

Diallertest wheat and milk atopic patches were tested on children aged one to thirteen years (N=123). Diallertest patches i.e. milk, wheat and control, were applied to the child's back either in the clinic or by their parents' at home. Once removed from the packaging, all three patches were identical, a pen was provided to inscribe the distinguishing mark (either M for milk, C for control or W for wheat) directly on the skin beneath each patch. Patches were removed after 48 hours and the initial photograph was taken. 24 hours later the second photograph was taken to record potential inflammation of the skin at either patch site where the active ingredient had been applied. Reactivity to APT was measured against milk and wheat oral challenge results previously conducted in a subset of the children and accessed from the clinic data base (N=6) or from the questionnaires (N=21). Oral challenges following an elimination diet are considered to be the gold standard in food intolerance diagnosis. The patch/oral challenge results were statistically analysed using SPSS15 to create chi-square contingency tables from which true and false positives and true and false negatives could be obtained. These results were further analysed into Positive Predictive Value (PPV) which is defined as the proportion of symptomatic individuals among test positives and Negative Predictive Value (NPV) which is defined as the proportion of non-symptomatic individuals among test negatives. Additionally, Sensitivity which is defined as the proportion of true positives detected and Specificity which is the proportion of true negatives detected, were obtained. (Niggemann et al, 1999). From these values overall agreement could be assessed. Overall agreement is defined as the total number of participants correctly classified by Diallertest divided by the total number of children tested.

The questionnaires were recycled from studies conducted over the previous four years from the Royal Prince Alfred Allergy Unit in regard to Autism Spectrum Disorder. The original questionnaires were condensed however, to encourage the parents involved in the Diallertest study to complete them and because some of the original material was deemed unnecessary for this trial. The condensed questionnaire included: Follow-up regarding dietary changes and common symptoms, this section also included oral challenge results, however, no information was requested in regard to the date that the challenge took place, nor the age of the child at the time of challenge. The questionnaire included the Connor's Rating Scale which is designed to assess autistic type behaviours/symptoms in children between the ages of 3 and

17. The third section is the PDDBI-C, (pervasive developmental disorder behavioural index-communication) and regards social interaction and communication skills. The final part is CEBAS, (children's eating behaviour and appetite scale). The questionnaires were supplied with a self-addressed, postage- paid envelope and once received were entered into the Allergy Unit's computer system along with the data from previous years. The children who were new to the study were given an I.D. number and entered into this system with the differentiation of non-ASD when this was the case. The information from the questionnaires could be grouped and readily analysed by using the SQL (structured query language) programme of IBM. We received training for this from the IT department at the Allergy Unit. Participant numbers and responses were then transferred into Excel and/or SPSS 15 (statistical package for Windows Version 15) for statistical analysis.

The diet history was taken from the attendant parent by either researcher (Gemma or Page), during the interview and Diallertest appointment.

Photographs were sent by the parents to Dr Velencia Soutter as e-mail attachments, although in some instances the photographs were received through the mail. The photographs were then entered onto the clinic data base. Agreement on each patch test result was then reached in discussion between Page, Gemma and Dr Velencia Soutter.

Results:

The major findings of the trial were:

Total persons successfully trialled who reacted to milk patch: 52% (46/88).

Total persons successfully trialled who reacted to wheat patch: 50% (42/84).

Total persons successfully trialled who reacted to both milk and wheat patch simultaneously:
33% (29/89).

Side effects:

One child, (male, four years), experience noticeable lethargy 2 hours after patch was applied, this was followed by loss of appetite and he awoke in the night with fever, the patches were removed 24 hours later and the child normalised within one hour. Pronounced urticaria subsequently appeared on his face and torso.

Comparison of Diallertest with Oral Challenge results:

While some of our results showed similar trends to those of previous studies (Niggemann et al, 1999, Canani et al, 2007, Fogg et al, 2006) the predictive value as demonstrated by overall agreement was poor.

Table 3. Comparison of RPA Diallertest results with APT/Oral challenge results previously reported:

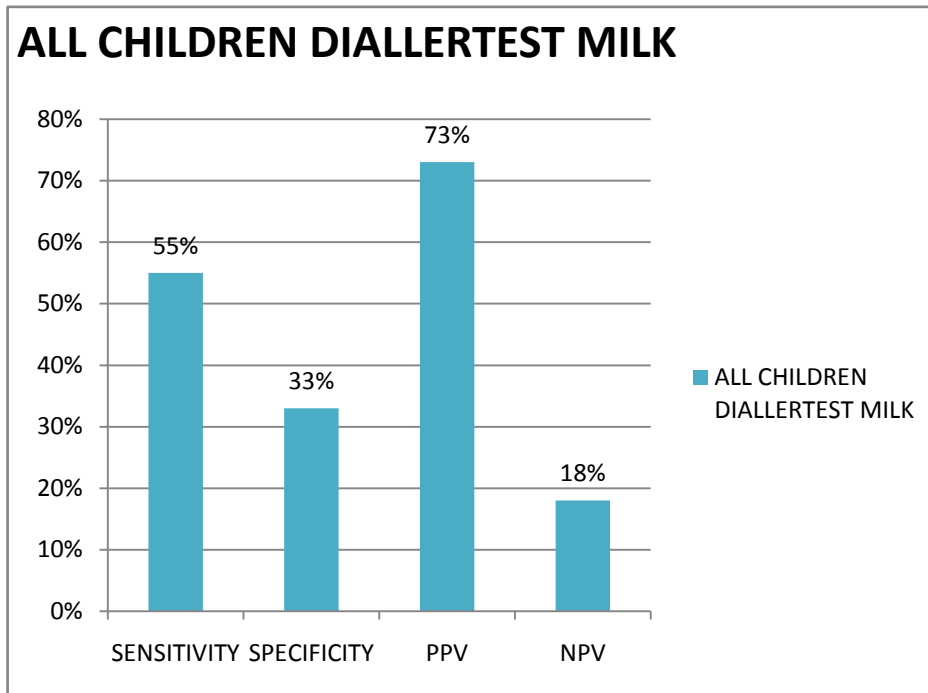
	Sensitivity	Specificity	PPV	NPV
Canani	6.4% CM	95.6% CM	66.6% CM	43.1%CM
Fogg	100%antigen	71%antigen	75%antigen	100%antigen
Niggeman	55% antigen	95% antigen	93% antigen	60% antigen
RPA Study	55% CM	33% CM	73% CM	18%CM

Code:

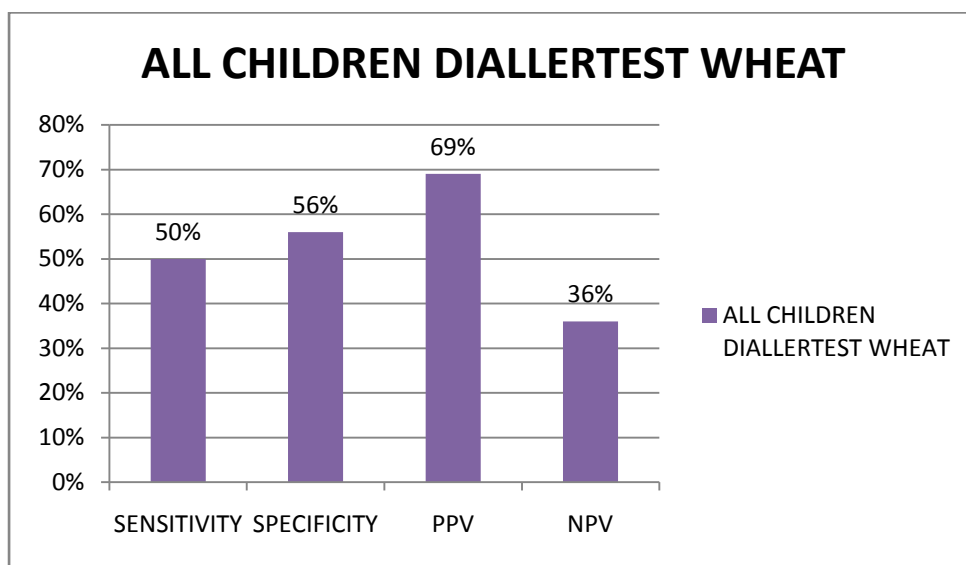
CM = cow's milk

antigen= specific food participant was known to react to.

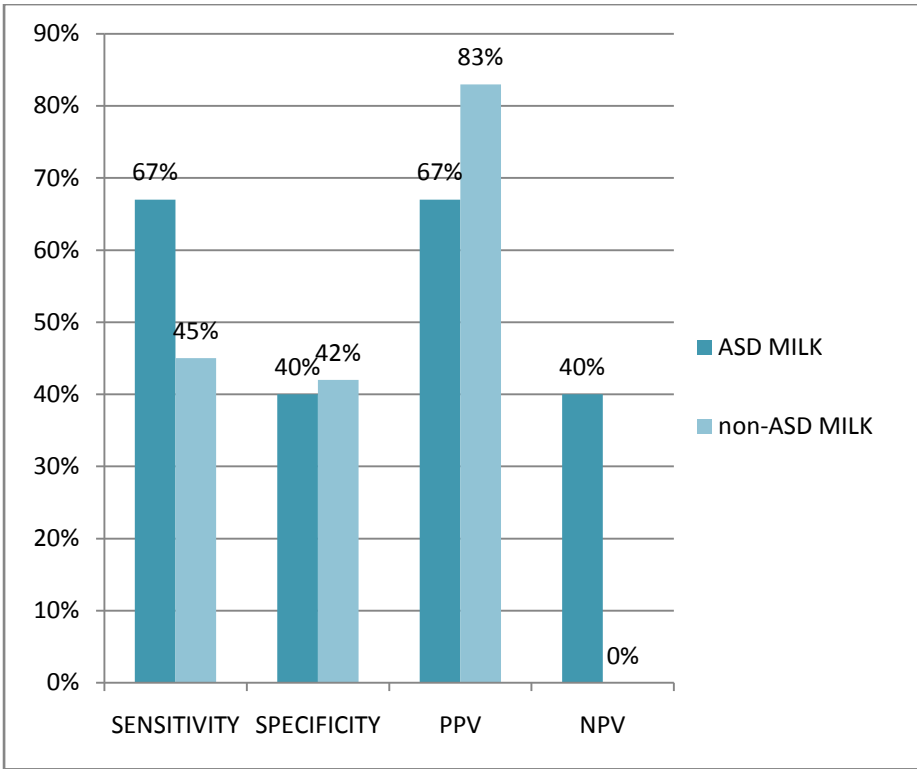
Comparison of Diallertest with Oral Challenge results as recorded by parents in questionnaire or as seen on Allergy Unit database:



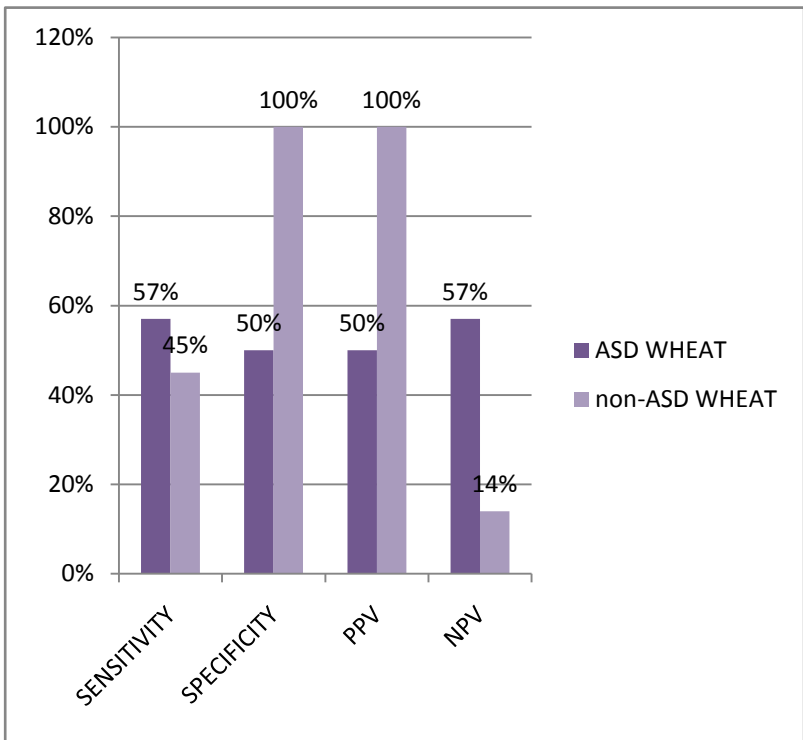
Graph 1. Results in Percentages All Children Milk.



Graph 2. Results in Percentages All Children Wheat.



Graph 3. Results in Percentages ASD and non-ASD Children Milk.



Graph 4. Results in Percentages ASD and non-ASD Children Wheat

Overall Agreement

All children milk 50%, All children wheat 52%, ASD milk 57%, Non-ASD milk 42%, ASD wheat 53%, Non-ASD wheat 50%.

Further Results

No significant correlations were observed between reactivity to patches and dietary restrictions i.e. elimination of either or both wheat and/or milk from regular diet.

No significant correlations were observed between reactivity to patches and gastrointestinal symptoms as reported in Follow up, Connors or CEBAS.

No significant differences were observed between the two groups, ASD and non-ASD in regard to patches, nor GIT symptoms.

A greater frequency of rhinitis and insomnia was noted in ASD children when comparing the two groups using the questionnaire.

Discussion:

Preliminary findings from this study suggest a limited predictive value for Diallertest wheat and milk patches in regard to oral challenge results. However, a limitation in this particular study is the interlude between the oral challenge and the patch testing, as changes in reactivity may have occurred during this time. Regrettably only 6 of the 31 oral challenge participants had had their oral challenges at RPA Allergy Unit. In one instance, the challenge had taken place two years earlier. Information on the remaining 25 participants was taken from the questionnaire and as previously noted, no dates or ages were given with respect to this information. In the literature, oral challenges had mostly been conducted within hours/days of determining patch test results and had taken place in either a clinical or hospitalised setting. One study which held the food challenges: “within two months of the APT, found that only 16 of 32 episodes that met the clinical criteria of Food Protein Induced Enterocolitis Syndrome were confirmed by challenge” and further suggested that: “it is likely that several of the patients lost reactivity to the suspected food in the interval between the onset of disease and the current study.” (Fogg et al, 2006). Another limitation of our study was that the mean age of the ASD participants was 8 years while the mean age of the comparative group was 5 years. Additionally, this was an open trial, no blinding occurred, it is therefore possible that reactivity could have been induced on a subliminal level.

Although no correlation could be found between reactivity and restricted diet or general gastrointestinal symptoms as reported in the Connors and CEBAS questionnaires, there was without doubt a high level of reactivity. For a total of successful Diallertest milk trials 52 % (46/88) of participants reacted. Likewise of the total successful wheat trials 50 % (42/84) of participants reacted positively to the patch.

The sheer number of reactors makes evidence of a detection process apparent, yet, the mechanism is poorly understood and questions arise as to

what, precisely, are the patches measuring? Could it partly be a response to lactase in the milk patches as this is a common food intolerance?

Presumably the wheat patches are recording gluten intolerance which could be readily proved in the celiac population. Furthermore could patch reactivity be simply “cellular memory” of a former intolerance once greater age/level of tolerance has been achieved, as was suggested by Fogg. (see above.)

It was also observed that of the ten children (N=89) who reacted to all three patches namely milk, wheat and control, six were known to have reflux, and two of the remainder had a history of ear infections, one of the remaining two had experienced a severe “whipworm infestation” two years previously. This relatively high incidence of ear, nose and throat sensitivity is reminiscent of a paper by Spergel, (2007) who reported a 77% resolution of symptoms in patients with pediatric oesophagitis who had eliminated foods indicated primarily by APT’s. Evidently, reactivity to the control patch is an anomaly and these results would normally be dismissed, however this hyper-reactivity/ENT pathology is intriguing.

A further observation was that none of these 10 “hyper-reactors” was from the ASD group. This factor may reflect findings of both Stubbs et al, (1976), and Warren et al, (1986) who noted that average T cell proliferative activity was significantly lower in autistic subjects. These reports led to the hypothesis that reduced T-cell responses may shape susceptibility to autism. (Altered T cell-mediated immunity and infectious factors in autism. Hu, Yong, Ph. D Utah State University, 1999.)

In this particular study it was anticipated that the comparative group would have a high level of sensitivity as the majority of these children were recruited from the RPA Allergy Unit and hence were known to be sensitive/reactive children. Additionally it has been reported by (Murch, 2005) that siblings of ASD children have a higher rate of allergy incidence than other children. Our study found that siblings of the ASD children had a much higher rate of reactivity to both patches 53% (9/17) than their ASD

siblings 27% (4/15). Reactivity to both patches by other children in the non-ASD group excluding the brothers and sisters was 31% (12/39) and reactivity to both patches by the total ASD group was 29% (10/35). Interestingly, the ASD children were more likely to react to one patch (either milk or wheat) than both their brothers and sisters : ASD 53% (8/15) vs Siblings 24% (4/17) or the total group ASD 43% (15/35) vs non-ASD minus siblings 28% (11/39). A report by Lisa Croen, suggested a two fold increase in autism among children of asthmatic mothers or women with allergies, particularly if their symptoms were medically diagnosed in the second trimester of pregnancy.

A study by “The Eurodiab Substudy 2 Study Group, 2000” reported that a prevalence of atopic dermatitis appears to afford some protection against Type 1 Diabetes Mellitus, an autoimmune condition. The authors hypothesized that autoimmunity tends to develop from a Th1-dominant immune response and that children exhibiting a Th2-dominant (IgE) response such as atopic dermatitis had seemingly evaded a more severe disease process.

It would be interesting to investigate whether this high level of atopy patch reactivity is common to siblings of ASD children in a larger cohort, if this *is* found to be the case one could surmise that this heightened mixed IgE/T-cell reactivity is symptomatic of a protective immune response against factors whereby their siblings (with ASD) could not mount a defence. It was shown in an animal model of diabetes (quoted in the study previously referred to) that inducing a shift from Th1 to Th2 responses prevents beta cell destruction. In the event that ASD is predominantly an autoimmune response and that myelin destruction is a major feature, a similarly induced shift, could, hypothetically, reduce damage to the CNS.

In a paper which appeared in “Immunology Today” in 1998, Rook, G and Stanford, J, suggested that if a vaccine prevents infection by using a Th2-mediated antibody-dependent mechanism, it may deprive the immune system of the learning experience that it would have derived from clearing

established infection within a Th1 mediated pathway. This of course, is a more sinister view regarding the Th1/Th2 paradigm, suggesting, as Andrew Wakefield (2000) did, that we have interfered excessively with natural immunity already, and that it is changes effectuated by childhood immunisation that have increased vulnerability to autism.

Conclusions:

Although our study was flawed by factors relating to the comparative measure it seems evident that Diallertest patches measured heightened reactivity in over 50 % of participants. Although we cannot clarify the predictive capacity of Diallertest at present neither can we dismiss the potential value of this product.

Digital photography was found to be an effective method for recording, conveying and storing results of atopy patch tests in clinical practice.

Siblings of ASD children appear to be more food sensitive/reactive to patch testing than children in the general population.

Children reacting to all 3 patches *may* be more inclined towards oesophageal irregularities.

Recommendations:

- Further analysis of Diallertest's predictive capacity in lactose intolerant and celiac populations.
- Exploration of the mechanisms at work in regard to Th1/Th2 in non-ASD siblings and their atopic reactivity.
- Questionnaires designed specifically to assess trends in the immune status of the ASD population.

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APPENDIX

Crosstabulation (Chi square Contingency Tables) of Diallertest/Oral challenge results:

Table i. DiallertestMILK * ChallengeMILK Crosstabulation

Count

		ChallengeMILK		Total
		Negative	Positive	Negative
DiallertestMILK	Negative	2	9	11
	Positive	4	11	15
Total		6	20	26

TABLE A. Working of Diallertest Milk Predictive Capacity in relation to Oral challenge.

Diallertest Milk Total Cohort	Challenge Milk Negative	Challenge Milk Positive	Total	
Negative	True negatives 2	False negatives 9	11	NPV 2/11
Positive	False positives 4	True positives 11	15	PPV 11/15
Total	6 Spec 2/6	20 Sens 11/20	26	OA 13/26

Table ii. DiallertestWHEAT * ChallengeWHEAT Crosstabulation

Count

		ChallengeWHEAT		Total
		Negative	Positive	Negative
DiallertestWHEAT	Negative	5	9	14
	Positive	4	9	13
Total		9	18	27

TABLE B. Working of Diallertest Wheat Predictive Capacity in relation to Oral challenge.

Diallertest Wheat Total Cohort	Challenge Wheat Negative	Challenge Wheat Positive	Total
Negative	True negatives 5	False negatives 9	14NPV 5/14
Positive	False positives 4	True positives 9	13PPV 9/13
Total	9 Spec 5/9	18 Sens 9/18	27OA 14/27

Table iii. DiallertestMILK * ChallengeMILK * ASD Crosstabulation

Count

ASD			ChallengeMILK		Total
			Negative	Positive	Negative
ASD	DiallertestMILK	Negative	2	3	5
		Positive	3	6	9
	Total		5	9	14
NonASD	DiallertestMILK	Negative	0	6	6
		Positive	1	5	6
	Total		1	11	12

TABLE C. Working of Diallertest ASD Milk Predictive Capacity in relation to Oral challenge.

ASD Diallertest Milk	Challenge Milk Negative	Challenge Milk Positive	Total
Negative	True negatives 2	False negatives 3	5 NPV 2/5
Positive	False positives 3	True positives 6	9 PPV 3/9
Total	5 Spec 2/5	9 Sens 6/9	14 OA 8/14

TABLE D. Working of Diallertest Non-ASD Milk Predictive Capacity in relation to Oral challenge.

Non-ASD Diallertest Milk	Challenge Milk Negative	Challenge Milk Positive	Total
Negative	True negatives	False	6 NPV 0/6

	0	negatives 6	
Positive	False positives 1	True positives 5	6 PPV 5/6
Total	1 Spec 0/1	11 Sens 5/11	12 OA 5/12

Table iv. DiallertestWHEAT * ChallengeWHEAT * ASD Crosstabulation

Count

ASD			ChallengeWHEAT		Total
			Negative	Positive	Negative
ASD	DiallertestWHEAT	Negative	4	3	7
		Positive	4	4	8
Total			8	7	15
NonASD	DiallertestWHEAT	Negative	1	6	7
		Positive	0	5	5
Total			1	11	12

TABLE E. Working of Diallertest ASD Wheat Predictive Capacity in relation to Oral challenge.

ASD Diallertest Wheat	Challenge Wheat Negative	Challenge Wheat Positive	Total
Negative	True negatives 4	False negatives 3	7 NPV 4/7
Positive	False positives 4	True positives 4	8 PPV 4/8
Total	8 Spec 4/8	7 Sens 4/7	15 OA 8/15

TABLE F. Working of Diallertest Non-ASD Wheat Predictive Capacity in relation to Oral challenge.

Non-ASD Diallertest Wheat	Challenge Wheat Negative	Challenge Wheat Positive	Total
Negative	True negatives 1	False negatives 6	5 NPV 1/5
Positive	False positives 0	True positives 5	12 PPV 5/5
Total	1 Spec 1/1	11 Sens 5/11	12 OA 6/12

