

5. Mehr S, Frith K, Barnes EH, Campbell DE, Group FS. Food protein-induced enterocolitis syndrome in Australia: a population-based study, 2012–2014. *J Allergy Clin Immunol*. 2017;140:1323–1330.
6. Feuille E, Menon NR, Huang F, Greenhawt M, Nowak-Wegrzyn A. Knowledge of food protein-induced enterocolitis syndrome among general pediatricians. *Ann Allergy Asthma Immunol*. 2017;119:291–292. e293.
7. Ludman S, Harmon M, Whiting D, du Toit G. Clinical presentation and referral characteristics of food protein-induced enterocolitis syndrome in the United Kingdom. *Ann Allergy Asthma Immunol*. 2014;113:290–294.
8. Sopo SM, Giorgio V, Dello Iacono I, Novembre E, Mori F, Onesimo R. A multicentre retrospective study of 66 Italian children with food protein-induced enterocolitis syndrome: different management for different phenotypes. *Clin Exp Allergy*. 2012;42:1257–1265.
9. Ruffner MA, Ruymann K, Barni S, Cianferoni A, Brown-Whitehorn T, Spergel JM. Food protein-induced enterocolitis syndrome: insights from review of a large referral population. *J Allergy Clin Immunol Pract*. 2013;1:343–349.

Within-tester repeatability and between-tester reproducibility of skin test endpoint titration

A quality assurance study



Airway response to allergen depends on 2 independent factors: the degree of immunoglobulin E sensitivity to the allergen and the level of lower airway response as quantified by histamine or methacholine challenge.^{1,2} The former can be assessed by skin prick titration (SPT) to determine the skin test endpoint (STE), which for research purposes is the smallest concentration of allergen that produces a skin wheal of approximately 2×2 mm (according to the standard operating procedure for the AllerGen NCE Clinical Investigator Collaborative). Allergen challenges are often performed in research settings when investigating a potential new pharmaceutical therapy for allergic asthma.^{3,4} The allergen concentration causing an immediate response (ie, provocative concentration of allergen causing a 20% decrease in forced expiratory volume in 1 second) can be predicted from the STE and the methacholine response; these data are routinely used to predict a starting allergen inhalation concentration that is safe and relatively time efficient.^{2–5} It is important to note that the STE is relevant only in the research context and has no bearing on clinical practice.

Given the reliance on the STE for the safe performance of allergen challenge testing, it is important to know the within-tester repeatability and between-tester reproducibility of STEs for quality assurance purposes. Only 1 study ($n = 7$) has examined repeatability in SPTs, which found limited insignificant variability in the STE.⁶

Seventeen individuals with allergies were recruited from the local community; 13 (8 men and 5 women; mean age 31 years) completed the study and 4 were excluded for lacking responsiveness to any of the study allergens. Antihistamines were withheld for an appropriate washout before testing.⁷ Informed consent was obtained before study commencement. This study was exempt from ethics approval because the biomedical research ethics board of the University of Saskatchewan defined this as a quality assurance study.

Participants with a history of SPTs in our laboratory or with a strong clinical history with 1 of the study allergens were not required to undergo preliminary skin prick testing. The study required 3 study visits, 1 with each of 3 testers separated by 1 week. Each visit involved the duplicate STE procedure performed according to the AllerGen NCE Clinical Investigator Collaborative standard operating procedure by a different tester in random order. If a participant was new to the laboratory, then the participant underwent skin prick testing during the first laboratory visit

according to European standards with a histamine positive control and a saline (0.9%) negative control.⁷

The standardized allergen extracts used for the SPTs were cat hair ($n = 10$; 10,000 BAU/mL; HollisterStier, Spokane, Washington), house dust mite *Dermatophagoides farinae* ($n = 2$; 30,000 AU/mL; HollisterStier), and mixed northern grass pollen ($n = 1$; 40,000 PNU/mL; Omega Labs, Montreal, Quebec, Canada). These extracts were diluted in 0.9% saline to make doubling dilutions from 1:8 to 1:1,024 (1:2,048 and 1:4,096 were made for 1 subject with high responsiveness to low concentrations). The same set of dilutions was used for each of a participant's visits. For each SPT, a saline control and increasing concentrations from 1:1,024 (from 1:4,096 in 1 subject) to 1:8 were applied in duplicate to a participant's forearm using a single-head metal lancet. After a 10-minute wait, the wheal sizes were measured at perpendicular angles to record the reaction.

For statistical analysis, the STE ratios were inverted (ie, 1:1,024 became 1,024) and logarithmically transformed. The use of dilution ratios normalized the participants' responses across the 3 study allergens, each of which had a different stock concentration. The resulting STEs were analyzed for within-tester repeatability through 2-way mixed intraclass correlation coefficients (ICCs) of each tester's duplicate STEs. One-way analysis of variance was performed using the smaller of the duplicate STEs measured by each tester for each participant to analyze the between-tester reproducibility. Significance was 2-sided and set at .05. The STE was defined as the allergen concentration causing a wheal no larger than 2×2 mm to maximize patient safety; it is safer to predict a lower starting concentration of allergen for inhalation testing than it is to induce a major allergic response with the first concentration inhaled. Sequence analysis was performed to exclude any potential tester order effect, including the possibility of loss of potency of older concentrations ($P = .9769$).

Each tester produced duplicate STEs with high absolute agreement (tester 1 ICC = 0.955; tester 2 ICC = 0.986; tester 3 ICC = 0.977), which reflects strong within-tester repeatability. In total, 28 of 39 SPTs resulted in identical duplicate STEs; only 1 duplicate measurement differed by 2 doubling dilutions and the remaining differed by only 1. Although the between-tester reproducibility of the overall sample was strong ($P = .3567$; Fig 1), the individual data present important considerations. Between-tester STEs were equivalent to or within 1 doubling concentration in 6 of 13 participants and within 3 doubling concentrations in 3 of 13 participants; however, in 4 of 13 participants, the smallest STE of at least 2 of the testers differed by more than 3 doubling concentrations; this difference is significant in research, because it could translate to the induction of an excessive allergic response in clinical trial participants. Typically, a safe starting concentration for an allergen challenge is

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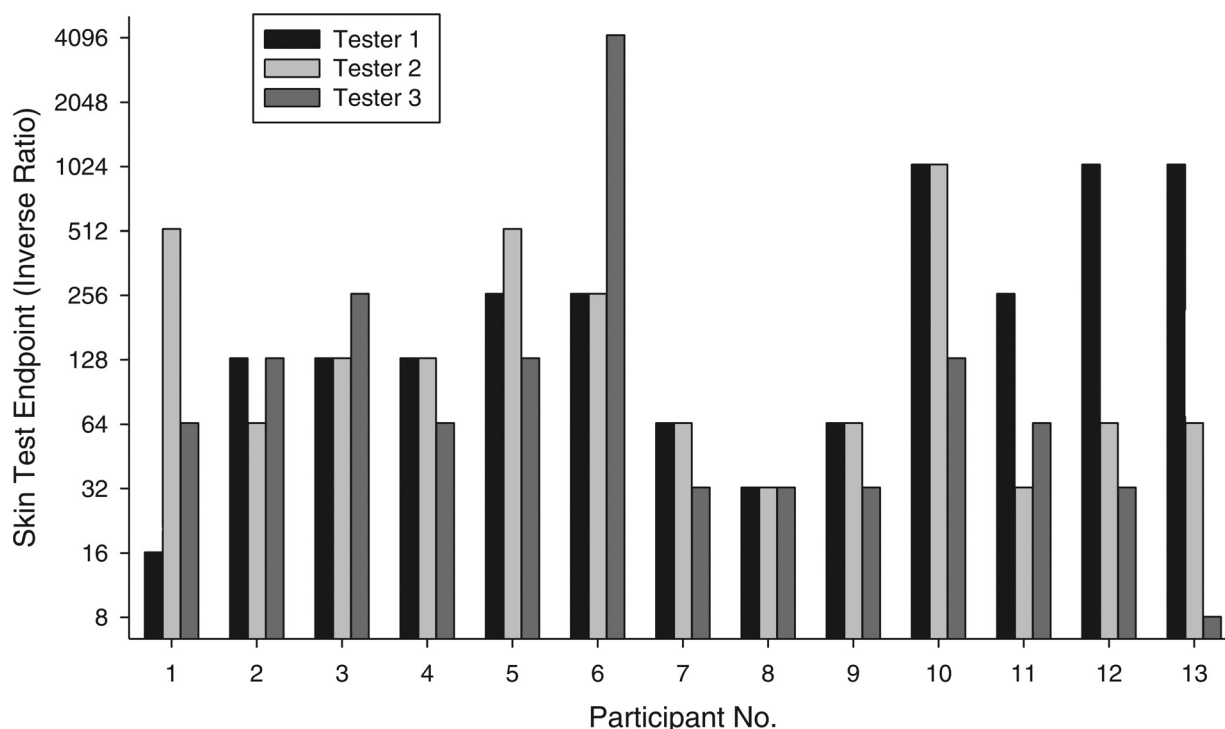


Figure 1. Skin test endpoints measured by each tester for each participant. Tester 1, novice; tester 2, 22 years of experience; tester 3, more than 40 years of experience.

determined as 3 doubling concentrations below the provocative concentration of allergen causing a 20% decrease in forced expiratory volume in 1 second predicted using the STE and the methacholine response.⁸

We can only speculate as to the cause of the difference in STEs of at least 3 doubling concentrations in 4 participants; 1 was briefly exposed to cat before his final test, which could have primed him for a higher dermatologic response to cat. To control for seasonal allergies, affected individuals were tested within that allergen season to keep the environmental factor as consistent as possible. The significant difference in STE could not be attributed to the experience level of the testers; tester 1 was a novice medical student, whereas tester 2 had 22 years of experience (PhD) and tester 3 had more than 40 years of experience (MD); no single tester was the source of most differences observed. It is unknown whether inter-tester differences or between-test factors are responsible for the high between-tester variance in STE in 4 of 13 participants.

From a quality assurance perspective, there is strong within-tester repeatability in SPTs; testing in duplicate should continue, because it is simple to perform and accounts for potential variability, thereby maximizing participant safety. Although there was strong between-tester reproducibility in STE results for 9 of 13 participants, the discrepancy between STEs in 4 of 13 participants raises concerns about such an occurrence during a clinical trial in which the risk is high (ie, potential for excessive response to inhaled allergen during allergen challenge testing). The mandatory 4-week washout from previous allergen exposures before SPTs and allergen challenges in clinical trials could help lessen the likelihood of this event. The STE is evidently the weakest link in the prediction equation for allergen responsiveness, because the other component of the prediction equation, methacholine

responsiveness, does not feature this level of discrepancy and has high sensitivity regardless of the tester involved.⁹ Future studies should consider methods for maximizing the accuracy of the prediction equation to best ensure the safe performance of the allergen challenge in pharmaceutical research.

Christianne M. Blais, MSc, MBA
 Beth E. Davis, PhD
 Donald W. Cockcroft, MD
 Division of Respiriology
 Critical Care and Sleep Medicine
 Department of Medicine
 University of Saskatchewan
 Saskatoon, Saskatchewan, Canada
don.cockcroft@usask.ca

References

- Cockcroft DW, Ruffin RE, Frith PA, et al. Determinants of allergen-induced asthma: dose of allergen, circulating IgE antibody concentration, and bronchial responsiveness to inhaled histamine. *Am Rev Respir Dis.* 1979;120:1053–1058.
- Cockcroft DW, Davis BE, Boulet LP, et al. The links between allergen skin test sensitivity, airway responsiveness and airway response to allergen. *Allergy.* 2005;60:56–59.
- Gauvreau GM, Arm JP, Boulet LP, et al. Efficacy and safety of multiple doses of QGE031 (ligelizumab) versus omalizumab and placebo in inhibiting allergen-induced early asthmatic responses. *J Allergy Clin Immunol.* 2016;138:1051–1059.
- Nomani S, Cockcroft DW, Davis BE. Allergen inhalation challenge, refractoriness and the effects of ibuprofen. *Allergy Asthma Clin Immunol.* 2016;12:24.
- Cockcroft DW, Murdock KY, Kirby J, Hargreave F. Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. *Am Rev Respir Dis.* 1987;135:264–267.
- Malling HJ. Reproducibility of skin sensitivity using a quantitative skin prick test. *Allergy.* 1985;40:400–404.
- Heinzerling L, Mari A, Bergmann K-C, et al. The skin prick test—European standards. *Clin Transl Allergy.* 2013;3:3.

8. Blais CM, Cockcroft DW, Davis BE. Allergen challenge testing in atopic asthma pharmaceutical research: Past, present and future directions. *EMJ Respir*. 2017;5:70–77.

9. Cockcroft DW, Davis BE. Direct and indirect challenges in the clinical assessment of asthma. *Ann Allergy Asthma Immunol*. 2009;103:363–370.

Coexistent *TBX1* mutation and chromosomal 20q13.13-q13.2 duplication in an infant with abnormal T-cell receptor rearrangement circle newborn screening results



An 8-day-old, term, male infant of a mother with diabetes and patent ductus arteriosus was transferred from an outlying facility for surgical management of truncus arteriosus (TA), coarctation of the aorta, hypoplastic aortic arch, and anomalous origin of the right subclavian artery. The newborn also had absent thymic shadow apparent on a chest radiograph, hypocalcemia, and abnormal newborn screening (NBS) results for severe combined immunodeficiency (SCID) measuring T-cell receptor rearrangement circles (TRECs); The threshold cycle was 36.86 (reference range, <36). Physical examination findings were notable for broad nasal bridge, retrognathia, ankyloglossia, and bilateral fifth digit clinodactyly. Family history was noteworthy for tetralogy of Fallot and isolated cleft lip and/or palate in cousins. No thymus was identified during cardiovascular surgery.

DiGeorge anomaly (DGA) secondary to a chromosome 22q11.2 microdeletion was suspected. Chromosomal microarray did not detect any deletions but rather identified a 3.79-Mb interstitial duplication of chromosome 20q13.13-q13.2. This region encompasses 6 genes (*PREX1*, *RNF114*, *UBE2V1*, *CEBPB*, *NFATC2*, *ADNP*) that may play a role in the immune system. However, the immunologic abnormalities associated with these genes are generally seen in the presence of deletions or mutations not duplications.

An immunologist was subsequently consulted because of an abnormal TREC NBS result. At time of consultation, the patient had recently begun treatment for extended-spectrum β -lactamase *Escherichia coli* tracheitis. Immunologic evaluation revealed lymphopenia and decreased CD3⁺, CD4⁺, CD8⁺, naïve CD4⁺CD45RA⁺ T cells, and CD56⁺ natural killer cells (Table 1). CD19⁺ B cell counts were normal. Lymphoproliferative responses to phytohemagglutinin, concanavalin A, and pokeweed mitogen were normal (Table 1). An XomeDx slice gene panel (GeneDx, Gaithersburg, MD), including *ADA*, *AK2*, *ATM*, *CD247*, *CD3D*, *CD3G*, *CHD7*, *CORO1A*, *DECLRE1C*, *DKC1*, *DOCK2*, *DOCK8*, *FOXN1*, *GATA2*, *IL2RG*, *IL7R*, *JAK3*, *LIG4*, *MTHFD1*, *MTR*, *NBN*, *NHEJ1*, *ORAI1*, *PNP*, *PRKDC*, *PTPRC*, *RAC2*, *RAG1*, *RAG2*, *SLC46A1*, *STAT5B*, *STIM1*, *TBX1*, and *ZAP70* mutations, was performed. Testing identified a previously unreported heterozygous mutation of *TBX1* (c.796 G>T, p.Glu266Ter [E266X]), which was believed to be a pathogenic variant consistent with a diagnosis of *TBX1*-related disorder. A different heterozygous likely pathogenic variant of *NHEJ1* (c.535_538delTTGA, p.Leu179ArgfsX15 [L179RfsX15]) was also identified. However, *NHEJ1*-related SCID is inherited in an autosomal-recessive manner, and a second mutation was not identified. Repeat measurement of TRECs at 3 weeks of age revealed normal counts (10,688 copies/10⁶ CD3⁺ T cells; reference range, >6794 copies/10⁶ CD3⁺ T cells; performed by Mayo Immunology Laboratory, Rochester, MN). Partial DGA was diagnosed in an infant with partial trisomy 20q13 syndrome.

Blanc et al¹ and Briand-Suleau et al² reported on the phenotype of 20q13.2 interstitial duplication, also known as partial trisomy 20q syndrome. The most common abnormalities are orofacial clefts, facial malformations, skeletal defects, cardiac malformations, and

neurodevelopmental delay. Facial phenotype includes large high forehead, wide nasal bridge, up-slanting palpebral fissures, anteverted nares, low-set ears, ear pits, branchial fistula, cleft lip and/or palate, protruding upper lip, prominent philtrum, and micrognathia. Cardiac abnormalities reported include ventricular septal defect, aortic coarctation, and hypoplastic left ventricle. Neurodevelopmental abnormalities are common but variable in severity. Musculoskeletal features include pectus excavatum, plagiocephaly, asymmetric and bisegmental vertebrate, scoliosis, and clinodactyly of the fifth fingers. No immunodeficiency has been associated with partial trisomy 20q syndrome. Our patient had broad nasal bridge and retrognathia, which may be present in both partial trisomy 20q syndrome and 22q11.2 microdeletion syndrome.

Up to 90% of DGA is attributable to heterozygous deletion of chromosome 22q11.2.^{3,4} DGA is a feature of the well-described 22q11.2 microdeletion syndrome, which is characterized by facial dysmorphism, including cleft palate and/or velopharyngeal insufficiency, conotruncal abnormalities, thymic hypoplasia, hypoparathyroidism, and hypocalcemia.^{5,6} Thymic hypoplasia or aplasia causes decreased T-cell maturation manifested as decreased numbers of mature and naïve T-cell numbers in DGA.^{5,6} DGA is classified as partial when associated with low but functional T cell counts. Chromosomal microarray analysis is considered a cost-effective and sensitive test for 22q11.2 deletion syndrome.^{3,4} Mutations of *TBX1* gene, encoded in the 22q11 region, have been implicated for causing DGA. Deletions of murine *Tbx1* have similar phenotypic features of DGA.⁵ In a case-control study by Yagi et al,⁷ *TBX1* gene mutation was found to be responsible for 5 patients who had DGA phenotype but no detectable deletion of 22q11.2 on genetic analysis. Other causes of DGA, including maternal diabetes, some teratogens, and other chromosome loci, have been described.

Although microarray analysis did not detect 22q11.2 deletion in this patient, the presence of TA, thymic hypoplasia, hypocalcemia, and lymphopenia were highly suspicious of DGA. Moreover, although abnormal TREC NBS results are associated with SCID, they may also be found in other patients,⁸ including those with decreased T cells (DGA, secondary T-cell lymphopenia), preterm infants, and patients with idiopathic T-cell lymphopenia. Furthermore, cytogenetic defects have also been reported with abnormal TREC NBS results.⁸ To ensure a known SCID mutation was not missed, an XomeDx slice gene panel was performed, and a heterozygous *TBX1* mutation was identified, confirming a diagnosis of DGA.

Although thymus was not identified in this patient, functional thymic tissue may have arrested in the neck in its descent into the anterior mediastinum.⁹ In a retrospective study by Crowley et al,¹⁰ 22q11.2 deletions that also included the *TBX1* gene region were associated with significantly lower CD3 and CD4 counts compared with deletions without *TBX1* involved. CD8, CD19, and CD56NK cell counts were similar among both groups.¹⁰ Bacterial tracheitis may be the cause or may have contributed to the findings of lymphopenia and decreased CD3⁺, CD4⁺, and CD56⁺ natural killer cells