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**Title:** Standardization, sensitivity and specificity of an ash (*Fraxinus excelsior*) pollen allergen extract

Frédéric 15564 de Blay frederic.deblay@chru-strasbourg.fr MD <sup>1</sup>, Ashok 15565 Purohit ashok.purohit@chru-strasbourg.fr MD <sup>1</sup>, Olivier 15566 Broutin obroutin@stallergenes.fr <sup>2</sup>, Agnès 15567 Viatte aviatte@stallergenes.fr <sup>2</sup>, Philippe 15581 Moingeon pmoingeon@stallergenes.fr <sup>3</sup> and Robert K. 15590 Zeldin rzeldin@stallergenes.fr MD <sup>2</sup>. <sup>1</sup> Unité de Pneumologie, Nouvel Hôpital Civil, Strasbourg, France, 67091 ; <sup>2</sup> Global Clinical Development, Stallergenes S.A., Antony, France, 92183 and <sup>3</sup> Scientific Department, Stallergenes S.A., Antony, France, 92183 .

**Body:** Background: Ash (*Fraxinus excelsior*), a wind-pollinated tree species causing spring time pollinosis, is the main representative of the Oleaceae family in temperate zones. Aims and objectives: There is a need to standardize allergen extracts. Here we calibrated the biological activity of an ash pollen in-house reference preparation (IHRP) in allergic subjects and assessed the sensitivity and specificity of a prick-test solution prepared from this IHRP. Methods: 27 ash pollen allergic subjects, with ash pollen and nOle e 1-specific serum IgEs (slgE) >2.0 kUA/L and >0.7 kUA/L, respectively and positive ash pollen nasal challenge tests (NCT) participated. Skin prick testing (SPT) with IHRP was performed and the concentration inducing a mean wheal diameter of 7 mm was defined as 100 IR/mL. Subsequently, a 100 IR/mL solution of IHRP was assessed in 30 ash allergic subjects (history of ash allergy and ash-specific slgE >0.7 kUA/L) and 30 non-allergic subjects (no history of allergy and ash-specific slgE <0.35 kUA/L). Results: The 100 IR/mL concentration corresponded to 1/148 weight/volume. All ash allergic subjects had a positive SPT (>3mm) and 29/30 non-allergic subjects had a negative SPT. Therefore, the sensitivity of the 100 IR/mL solution was 100% [88.6-100.0] and its specificity was 96.7% [83.3-99.4]. Conclusions: A 100 IR/mL prick test solution for in vivo diagnosis of ash pollen allergy was shown to be highly sensitive and specific.