Converting Allergen Extract Strength Units: Protein Nitrogen Units (PNU) and Weight-to-Volume (W/V)

ALK TECHNICAL MEMO
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ALK Medical Scientific Affairs

ALK has been in the allergenic extract market for more than 95 years. Since entering the US market in 1985, ALK has grown to be one of the top US extract suppliers, providing consistent, quality products to numerous markets offering allergy testing and treatment.

When transitioning to a new allergen formulation, there is much to take into consideration. It is essential to understand the appropriate target dosing and maintain patient safety for a smooth transition. ALK has transitioned many practices between allergen extract formulations, and is dedicated to providing the necessary resources to alleviate concerns that may arise during the process. This technical memo is provided as a guidance document to assist you with conversion between non-standardized allergen extract formulations. Additionally, our Medical Scientific Affairs Team is available to personally assist you during this transition and answer any questions you may have. We are dedicated to ensuring that your transition is as seamless as possible.

Non-standardized Allergen Extract Formulations

While allergenic extracts are available in multiple formulations and strengths, all are generated by combining source material (e.g., pollen, mold, etc.) with set volumes of solutions designed for extracting allergen proteins. Weight-to-volume (w/v) extracts result from this extraction process. These extracts carry the designation of 1:10 w/v or 1:20 w/v, referring to the ratio of raw material extracted in solution. These w/v extracts may also be adjusted to a certain Protein Nitrogen Unit (PNU) and sold at specific concentrations. Each formulation has unique advantages, and both are successfully utilized for immunotherapy nationwide. In order to determine which formulation is best for your specific practice, we have outlined the differences between PNU and w/v extracts below.

Weight-to-Volume (w/v)

Most w/v extracts are generated at the 1:10 w/v aqueous strength. This formulation serves as the mother lot from which most other extract formulations are derived. Glycerin extracts may be generated two ways: 1) addition of equal parts of 100% glycerin to the aqueous mother lot, yielding a 1:20 w/v extract; or 2) direct extraction using 50% glycerin solutions. The concentration of w/v extracts is typically much higher than diluted, PNU formulations, conferring greater protein stability for long-term storage. This equates to more stable potency over the lifespan of the product. Many physicians prefer the use of w/v dosing, as outlined in the Allergy Practice Parameters, as it negates the need for PNU dilution calculations. If needed, our Medical Scientific Affairs Team has information available regarding the w/v target dosing outlined in the Allergen Immunotherapy Practice Parameters Update[1].

Protein Nitrogen Unit (PNU)

PNU was one of the first measurements used to describe extract strength and indicates the concentration of total protein in a vial. PNU identifies the total nitrogen present in a sample measured by the Kjeldahl assay, as specified by the FDA. Nearly all aqueous extracts are subjected to PNU determination as part of FDA-required quality control measures, therefore this measurement is also used to dilute extracts to specific PNU concentrations (i.e. 10,000, 20,000, and 40,000 PNU/mL). These dilutions are useful for formulating immunotherapy dosage by PNU.

There are a few issues to note when using PNU for immunotherapy applications. Not all of the components identified by the Kjeldahl assay are relevant allergenic proteins, therefore the PNU concentration on the label may overstate the actual amount of true allergen in the vial. To further complicate the issue, studies have shown that in some cases not all of an allergen protein is identified by Kjeldahl assay, causing PNU to understate allergenic content. May et al. reported that only roughly half of the short ragweed in solution was captured by Nitrogen determination[2]. Because of this, the FDA does not recognize PNU as a potency measurement and therefore is not a reliable parameter for standardization. Nevertheless, knowledge of PNU concentration does provide some useful information, and is often utilized as a unit for therapeutic dosing.

Converting between PNU and w/v Extracts

Transitioning between PNU and w/v extracts is a relatively straight-forward process, but does require two simple calculations. Before getting started, acquire the PNU value of the w/v extract that will be diluted, identify the desired (diluted) PNU concentration, and determine the total volume of diluted extract that will be created. Most aqueous extracts state the PNU concentration on the vial label. For glycerin extracts or those extracts without a labeled PNU concentration, contact ALKís Medical Scientific Affairs Team for the historical PNU concentration via Science@alk.net.

To determine how much w/v extract is needed to achieve a desired PNU concentration, use the formula below:

Volume of w/v extract needed =
(desired PNU ÷ PNU of w/v extract) X Total vial volume

Divide the desired PNU concentration by the PNU concentration of the w/v extract; multiply the resulting number by the total volume of diluted extract needed.

Example : (10,000PNU/mL desired ˜ 80,000PNU/mL w/v extract) X 10mL = 1.25mL of w/v extract needed.

Next, determine how much diluent is needed to dilute the extract above in a new vial:

Volume of diluent needed =
Total vial volume – Volume of w/v extract needed (determined above)

Subtract the volume of w/v extract needed (answer from previous calculation) from the total vial volume that will be created (i.e. 10mL in above example).

Example: 10mL ñ 1.25mL = 8.75mL of diluent

Finally, use the two numbers from the formulas above to create a diluted extract in a sterile empty vial by combining the w/v extract with diluent.

It is important to note that when the desired PNU concentration is greater than the w/v extract PNU concentration, a dilution cannot be made. For instance, Mountain Cedar 1:10 W/V typically has a PNU concentration below 10,000. Therefore a 20,000 PNU/mL vial cannot be formulated. When issues such as these arise, feel free to contact Medical Scientific Affairs for advice on extract formulation.

Safety Precautions

Recommendations for transition can be safely made using standard of care practices detailed in the Allergy Practice Parameters, published by the American Academy of Asthma, Allergy and Immunology (AAAAI)[1]. When converting allergen dosage from one extract lot to another, a 50-75% dose reduction is encouraged to reduce the potential for an adverse reaction; this reduction is applied to the previous injection volume (e.g., if 0.5 cc is being given as a maintenance injection, reduce dosage volume to 0.25 or 0.13 cc). If no reaction occurs, dosage can be increased, incrementally, back to the dose injected prior to conversion (e.g., 0.25, 0.35 or 0.5 cc). The incremental increase in dosage utilized should correspond to the build-up regimen you are currently employing. In extremely sensitive patients, a 90% dose reduction can be implemented as a precautionary measure.

ALK Commitment

ALK is committed to helping Allergy Specialists maintain uniformity of care for their patients. Please do not hesitate to contact your Allergy Consultant, Customer Service (800.325.7354) or Medical Scientific Affairs (855.782.9323, science@alk.net, or submit your scientific questions to our 24/7 online helpdesk in a support ticket at: https://alkinc.freshdesk.com) should you have additional questions or concerns regarding a transition between ALK products.

This technical memo is not intended to replace physician judgment with respect to the clinical diagnosis and treatment of patients. All decisions regarding potential patient care are solely at the discretion of the treating physician.

References

1. Cox L, Nelson H, Lockey R, et al. Allergen immunotherapy: a practice parameter third update. *J Allergy Clin Immunol*. 2011;127(1 Suppl):S1-S55.
2. May JC, Sih JT, Miller JR, Seligmann EB Jr. Optimization of parameters in protein nitrogen unit precipitation procedure for allergenic extracts. *J Allergy Clin Immunol*. 1979;63(2):87-97.