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Published in:
Toxicon

Link to article, DOI:
[10.1016/j.toxicon.2018.05.021](https://doi.org/10.1016/j.toxicon.2018.05.021)

Publication date:
2018

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):

Laustsen, A. H. (2018). Toxin-centric development approach for next-generation antivenoms. *Toxicon*, 150, 195-197. <https://doi.org/10.1016/j.toxicon.2018.05.021>

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Accepted Manuscript

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PII: S0041-0101(18)30210-1

DOI: [10.1016/j.toxicon.2018.05.021](https://doi.org/10.1016/j.toxicon.2018.05.021)

Reference: TOXCON 5902

To appear in: *Toxicon*

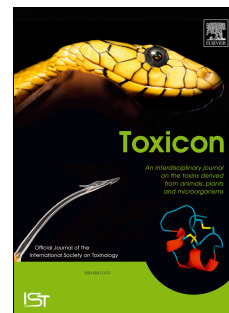
Received Date: 19 April 2018

Revised Date: 17 May 2018

Accepted Date: 28 May 2018

Please cite this article as: Laustsen, A.H., Toxin-centric development approach for next-generation antivenoms, *Toxicon* (2018), doi: 10.1016/j.toxicon.2018.05.021.

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2 Toxin-centric development approach for next-generation antivenoms

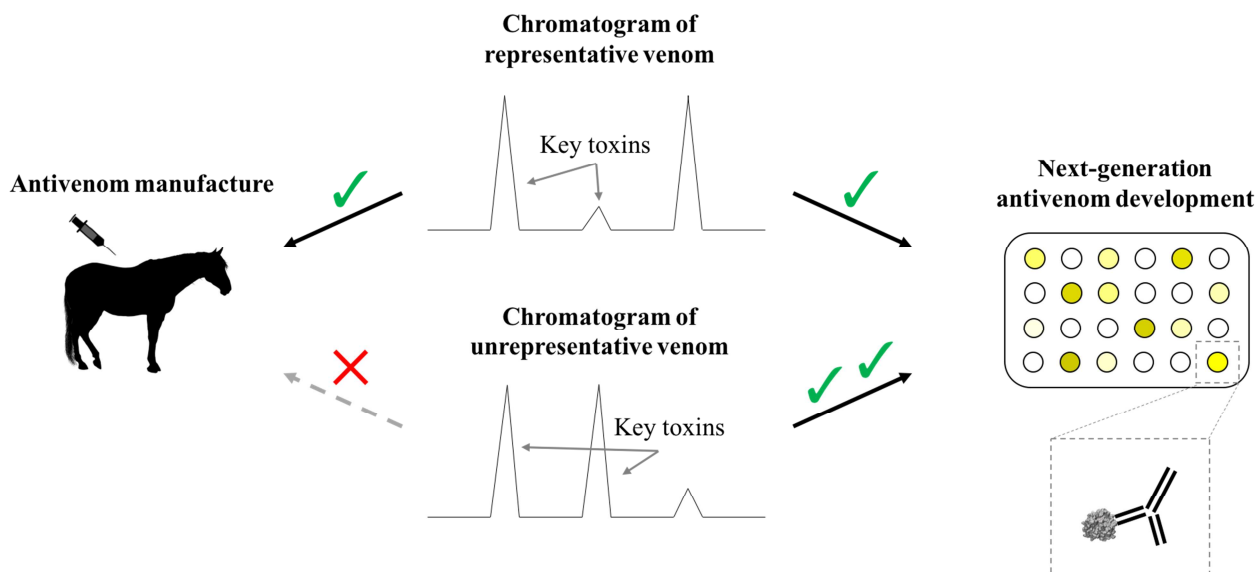
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4 With its recent re-introduction on the World Health Organization's list of Neglected Tropical
5 Diseases (Chippaux, 2017), snakebite envenoming is gaining increased international attention. Not
6 only does this create hope that new policies and guidelines may help healthcare systems to
7 implement better preventive strategies, but it may also create incentive for research and
8 development efforts aiming at delivering new antivenom products for treatment of snakebite
9 envenoming. New products may fall into two categories: 1) Plasma-derived antivenoms based on
10 conventional immunization approaches (Gutiérrez et al., 2011; World Health Organisation, 2010)
11 and 2) next-generation antivenoms based on specific therapeutic molecules that are selected on their
12 ability to neutralize key toxins (Knudsen and Laustsen, 2018), and which are manufactured without
13 the need to procure snake venoms. The benefit of the first type of antivenom products is that their
14 therapeutic benefits have been historically documented (Gutiérrez et al., 2011). However, they
15 suffer from the drawback of not being compatible with the human immune system due to their
16 heterologous nature (Laustsen et al., 2016a), and from having a low content of therapeutically
17 active antibodies, particularly against small venom components with low immunogenicity and high
18 toxicity (Laustsen et al., 2017; Leong et al., 2015; Tan et al., 2015, 2016). In recent time, venomics
19 and antivenomics approaches have helped build a greater understanding of the antigenicity of
20 different venom components, which may provide guidance on how to design immunization
21 mixtures that give rise to venom-paraspecific antibody responses in production animals (Calvete et
22 al., 2014, 2009; Lomonte and Calvete, 2017). Such knowledge creates a foundation for improving
23 the neutralization potential of antivenoms against toxin subfamilies with low immunogenicity, such

24 as small neurotoxins from the three-finger toxin family (Calvete et al., 2018; Tan et al., 2017). In
25 contrast to plasma-derived antivenoms, the development of next-generation antivenoms is
26 unaffected by any discrepancy between toxicity and immunogenicity, as monoclonal antibodies and
27 small molecule inhibitors can be discovered even for non-immunogenic toxins.

28 Independent on the particular molecular scaffold (small molecule, antibody, antibody
29 fragment etc.) employed in a next-generation antivenom, a fundamental change in our
30 understanding of snake venoms as drug targets will have to occur. Conventional antivenom
31 manufacture involves the use of whole venoms during the immunization process. For this approach,
32 it is important to optimize the immunization mixture to ensure that the venoms employed are
33 representative of the venoms in the geographic region the antivenom is to be deployed (Gutiérrez,
34 2007). This necessitates the collection of snakes with venom compositions that are representative of
35 the given region, as such venom compositions have been shown to vary quite dramatically across
36 geographic ranges (Chippaux et al., 1991). Among other things, this possibly creates an incentive
37 for establishing serpentaria in different regions of the world.

38 In contrast to conventional antivenom manufacture, the manufacture of next-
39 generation antivenoms installs a very different demand, as these antivenoms are not dependent on
40 snake venoms for their manufacture (Laustsen et al., 2016b). A thorough understanding of venom
41 compositions in relation to geographic region is still essential to guide the compositional
42 formulation of next-generation antivenoms (Laustsen, In press), but the need to procure snake
43 venoms in large quantities becomes irrelevant. In fact, the development of specific molecular
44 antitoxins is not even reliant on ‘representative’ whole venoms, if the key toxins can be isolated
45 from a given venom in sufficient quantities for antitoxin discovery purposes (Figure 1).

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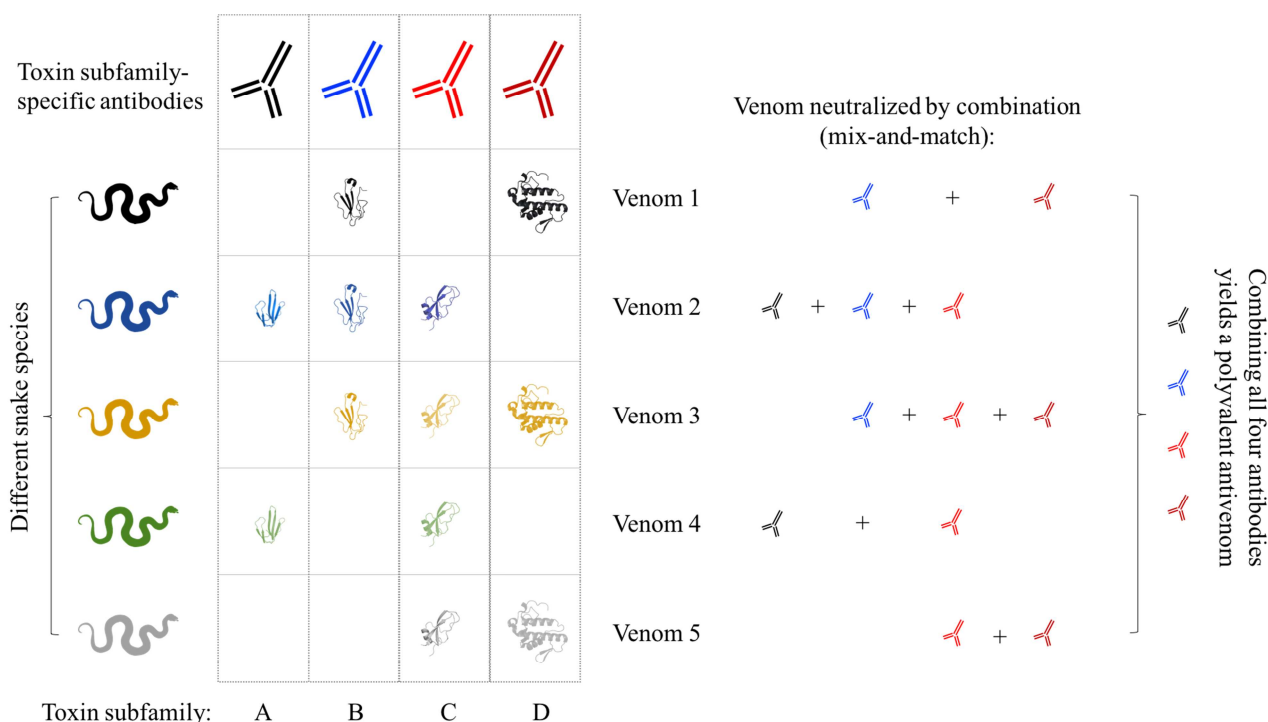
48 **Figure 1.** Schematic representation of chromatograms for two different venoms from the same
 49 snake species (one venom that is representative of the venom compositions of snakes in a given
 50 geographic region and one venom that is not). For antivenom manufacture, it is important that the
 51 venoms employed in the immunization mixture are representative of the venoms in the geographic
 52 region that the antivenom is to be deployed. In comparison, a venom does not need to be
 53 representative when used for next-generation antivenom development purposes, as the key toxins
 54 typically are to be isolated and used in pure form. An unrepresentative venom with high
 55 proportions of key toxins will even be more useful than a representative venom with lower
 56 proportions.

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58 The exact timing of when next-generation antivenoms will be introduced in the clinic
 59 is unknown. However, the transition from plasma-derived antivenoms to antivenoms based on
 60 defined mixtures of synthetic and/or recombinant components seems unlikely not to happen in the
 61 future (Laustsen et al., 2016a). With this transition, the need remains for studying snake venom
 62 compositions and identifying all toxins that are medically relevant to neutralize (Lomonte and
 63 Calvete, 2017), but the focus in antivenom development will indeed change from obtainment of

64 whole venoms that are representative of certain habitats, age groups, and geographical regions to a
65 more toxin-centric focus, where it is more important to obtain key snake toxins in isolated form
66 than it is to procure representative venoms. With the toxin-centric focus in antivenom development,
67 antitoxin discovery efforts should not aim to identify molecular antitoxins that can target a group of
68 toxins within only one whole venom, but rather focus on neutralization of toxin subfamilies across
69 multiple species and identification of which (conserved) toxin epitopes should be targeted
70 (Engmark et al., 2016, 2017b; Laustsen, In press). This will allow for a mix-and-match approach,
71 where polyvalent next-generation antivenoms can be built up by mixing individual toxin subfamily-
72 specific antitoxins that combined may neutralize all the key toxin subfamilies in several target
73 venoms (Figure 2). It may therefore become more relevant to identify key toxins from different
74 toxin subfamilies that are essential to be neutralized than to identify whole venoms that are
75 representative for a given species (in a given geographic region). In this context, it may also be
76 relevant to explore how broad target specificity can be obtained for different antitoxins, as it will
77 likely be difficult to identify single antitoxins that can cross-neutralize an entire subfamily of toxins
78 (Engmark et al., 2017a), although promising results have been reported for certain inhibitors against
79 enzymatic toxins (Arias et al., 2017; Knudsen and Laustsen, 2018; Lewin et al., 2016).

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82 **Figure 2:** Schematic overview of how polyvalent next-generation antivenoms can be designed by
 83 combining antitoxins (illustrated with IgG antibodies) that can cross-neutralize toxins from the
 84 same subfamily across different snake species. This approach requires that focus in antivenom
 85 development is shifted from representative whole venoms from specific snake species to becoming
 86 more toxin-centric.

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If these predictions hold true, the implications may firstly become significant for governments and healthcare systems that are considering the establishment of new serpentaria to obtain collections of snake specimens with venom compositions that are representative for their geographical region, as such serpentaria risk becoming redundant. Secondly, antivenom researchers may have to re-evaluate how they understand the interplay between toxins and venoms and switch from a currently strong focus on neutralizing whole venoms towards a focus on neutralization of toxin subfamilies across multiple snake species.

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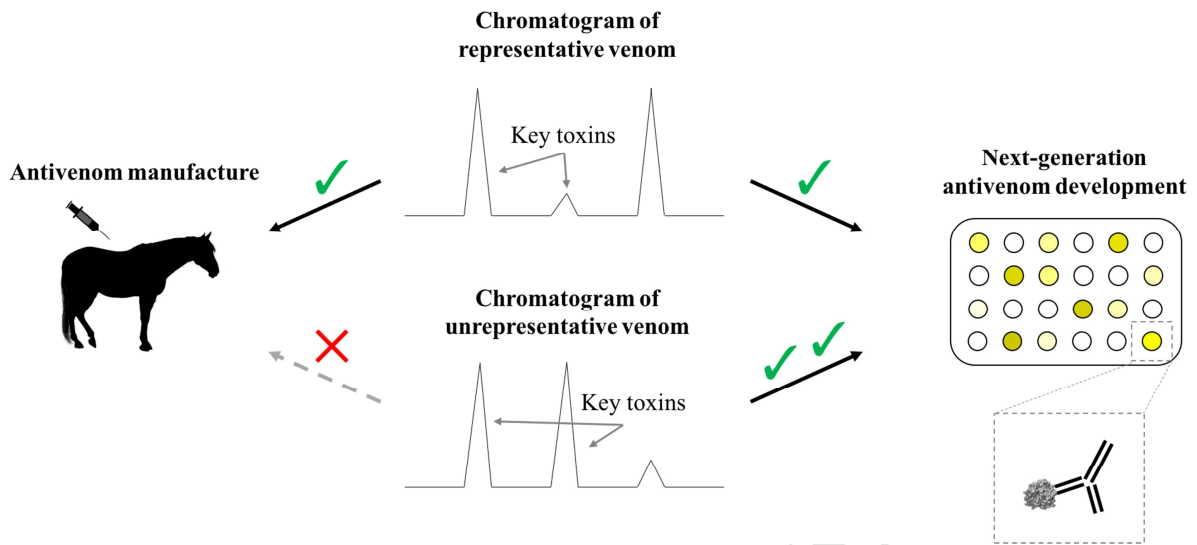
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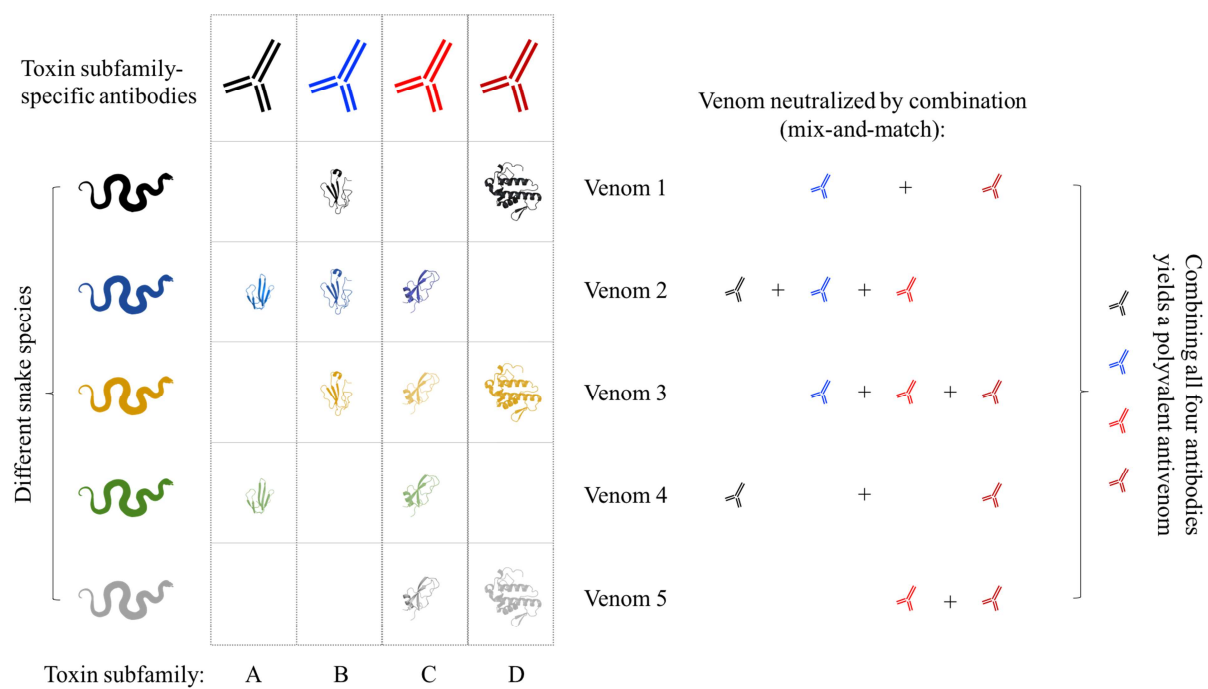
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Conflicts of Interest: The author declares no conflict of interest.

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