Title: Disease Bundling or Specimen Bundling? Cost- and Capacity-Efficient Strategies for Multi-disease Testing with Genetic Assays

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A Online Appendix

A.1 Summary of Notation

We denote vectors in boldface, random variables in upper-case and their realization in lower-case letters, use ";" for probabilistic conditioning, and provide the arguments of a function in parantheses.

The disease set $N = \{1, 2, \cdots, n\}$ is arranged in non-increasing order of disease prevalences $\pi_i, i \in N$, that is, $\pi_1 \geq \pi_2 \cdots \geq \pi_n$. The *joint* vector, $\boldsymbol{p}$, contains all mono-, co-, and no-infection probabilities for the n diseases, and the *marginal* vector $\boldsymbol{\pi} = (\pi_i)_{i \in N}$ contains the disease prevalences (Definition 1). $\boldsymbol{S} = (S^k)_{k=1,\cdots,q}$, $q = 1, \cdots, n$, denotes a q-partition of the disease set N, where each assay S^k has cardinality (size) s^k, prevalence $\pi(S^k)$, and pool size t^k, with $t^{*k}(S^k) = t^{*k}(\pi(S^k))$ denoting an optimal pool size within the domain $t \in Z^+, t \geq 1$ (with $t = 1$ denoting individual testing), which is a function of assay prevalence $\pi(S^k)$ only (Property 1). When referring to an optimal Dorfman pool size, we use the notation $t^{*k}_D(\pi(S^k))$, that is, considering the domain $t \in Z^+, t \geq 2$. We use the terms partition and assay portfolio interchangeably. In general, we use the superscripts k, k', l for assay (with a subscript added to the index in places, e.g., k_1, k_2), and the subscripts i, j, r for disease.

Functions $T(S, t)$ and $C(S, t)$ respectively denote the per subject expected number of tests ("expected tests") and per subject expected testing cost ("expected testing cost") for assay S with pool size t, and $TC(\boldsymbol{S}, \boldsymbol{t}, \lambda)$ denotes the expected total testing cost ("total cost") for partition $\boldsymbol{S}$, pool size vector $\boldsymbol{t}$, and coefficient $\lambda \in [0, 1]$, where λ (hence, $1 - \lambda$) is the weight of the expected testing cost (expected tests) in the objective function.

By Definition 3, $D^{(q)}$ and $I^{(q)}$, $q = 1, \cdots, n$, and $M^{(q)}$, $q = 2, \cdots, n$, respectively denote the *optimal* testing design when constrained to be within the q-partitioned Dorfman, individual-testing, and mixed-testing design classes (an $M^{(1)}$ design is not possible). When needed, we use the design class as a subscript to refer to the corresponding metric for the optimal design within that design class, e.g., $\boldsymbol{S}_{D^{(q)}}$, $T_{D^{(q)}}$, and $TC_{D^{(q)}}$ respectively denote the partition, the expected tests, and the total cost for an optimal $D^{(q)}$ design.

The composite cost function is given by $\tilde{c}(s, \lambda) = \lambda\ c(s) + 1 - \lambda, \forall s \in Z^+, \lambda \in [0, 1]$, where $c(.)$ is the assay cost function; by definition, any $\tilde{c}(.) \in \tilde{C}$ must satisfy Assumption $(\boldsymbol{A})$. When needed, we use the notation that composite cost functions $\tilde{c}(.)$ and $\tilde{c}'(.)$ respectively utilize assay cost functions $c(.)$ and $c'(.)$. The notation, $\tilde{c}(.) \geq_{diff} \tilde{c}'(.)$, denotes that function $\tilde{c}(.)$ has higher differences than function $\tilde{c}'(.)$ (Definition 5).

To simplify the notation, we drop the indices and function arguments when clear from context, e.g., $\tilde{c}(s)$ for $\tilde{c}(s, \lambda)$ when λ does not change, or $\tilde{c}(.)$ when referring to a generic composite cost function, $\boldsymbol{S}$ for $\boldsymbol{S}_{D^{(q)}}$ when it is clear from context that we are referring to an optimal partition for the $D^{(q)}$ design class. We also use the notation that $T^*(S) = T^*(\pi(S)) = T(\pi(S), t^*(\pi(S)))$ and $T^*_D(S) = T^*_D(\pi(S)) = T(\pi(S), t^*_D(\pi(S)))$, that is, expected tests for assay S at the (global) optimal pool size, and at the optimal Dorfman pool size, respectively.

A.2 Preliminaries

By Definition 3, optimal pool size vectors for the different design classes are given by, $\boldsymbol{t}_{I^{(q)}} = \boldsymbol{1}$, $\boldsymbol{t}_{D^{(q)}} \geq \boldsymbol{2}$, and $\boldsymbol{t}_{M^{(q)}} : \exists k, l = 1, \cdots, q : t^k_{M^{(q)}} = 1,\ t^l_{M^{(q)}} \geq 2$. Any assay with individual testing ($t = 1$) uses one test per subject for all the diseases in the assay, i.e., $T(S, 1) = 1, \forall S \subseteq N$ (Eq. (8)).

Then, by Eq. (2), the total cost of the (global) **TD** optimal solution (denoted by superscript *), and the optimal solution when constrained to be within each design class (denoted by the design class subscript), follow:

$$TC^*(\lambda) = \quad TC(\boldsymbol{S}^*, \boldsymbol{t}^*, \lambda) \ = \ \sum_{k=1}^{q^*} \tilde{c}(s^{k*}, \lambda) \times T^*(S^{k*}), \quad \text{where } q^* \in \{1, \cdots, n\} \tag{17}$$

$$TC_{I^{(q)}}(\lambda) \ = \ \sum_{k=1}^{q} \tilde{c}(s^k_{I^{(q)}}, \lambda) \times T(S^k_{I^{(q)}}, 1) = \sum_{k=1}^{q} \tilde{c}(s^k_{I^{(q)}}, \lambda) \times 1 = \sum_{k=1}^{q} \tilde{c}(s^k_{I^{(q)}}, \lambda), \quad \forall q = 1, \cdots, n \tag{18}$$

$$TC_{D^{(q)}}(\lambda) \ = \ \sum_{k=1}^{q} \tilde{c}(s^k_{D^{(q)}}, \lambda) \times T^*_D(S^k_{D^{(q)}}) = \sum_{k=1}^{q} \tilde{c}(s^k_{D^{(q)}}, \lambda) \times \left[\frac{1}{t^{k*}_{D^{(q)}}} + 1 - \left(1 - \pi(S^k_{D^{(q)}})\right)^{t^{k*}_{D^{(q)}}} \right], \quad \forall q = 1, \cdots, n \tag{19}$$

$$TC_{M^{(q)}}(\lambda) \ = \ \sum_{k=1..q : t^k_{M^{(q)}} = 1} \tilde{c}(s^k_{M^{(q)}}, \lambda) \times T(S^k_{M^{(q)}}, 1) + \sum_{l=1..q : t^l_{M^{(q)}} \geq 2} \tilde{c}(s^l_{M^{(q)}}, \lambda) \times T^*_D(S^l_{M^{(q)}}). \tag{20}$$

The following definition and properties will be used subsequently in the proofs.

Definition A1. *1. Function $f(.)$ is a subadditive real-valued function if $f(x+y) \leq f(x)+f(y)$, $\forall x, y \in \mathbb{R}$ [55]. 2. Function $f(.)$ is a subadditive set function if $f(S^1 \cup S^2) \leq f(S^1)+f(S^2)$, for any pair of sets S^1, S^2 [45].*

Property A.1. *(1) $T^*(\pi(S))$ and $T^*_D(\pi(S))$ are subadditive in $\pi(S) \in [0, \underline{p}]$. (2) Both $c(s)$ and $\tilde{c}(s,\lambda)$ are subadditive in $s \in Z^+$, $\forall \lambda \in [0,1]$. (3) $\pi(S^1 \cup S^2)$ is a subadditive set function, $\forall S^1, S^2 \subseteq N$.*

Proof. $T^*_D(\pi(S))$ is non-negative and concave increasing in $\pi(S) \in [0, \underline{p}]$, and $T^*(\pi(S)) = T^*_D(\pi(S))$ for $\pi(S) \in [0, \underline{p}]$ (Property 2). Similarly, $c(s)$ is non-negative and concave non-decreasing in $s, \forall s \in Z^+$ (Assumption ($\boldsymbol{A}$)), and $\tilde{c}(s,\lambda) = \lambda\ c(s) + 1 - \lambda$ is a linear function of $c(s)$, $\forall \lambda \in [0,1]$; and for any two sets $S^1, S^2 \subseteq N$, $\pi(S^1 \cup S^2) = \pi(S^1) + \pi(S^2) - \pi(S^1 \cap S^2) \leq \pi(S^1) + \pi(S^2)$. Then, the subadditivity results follow by Definition A1. □

Property A.2. *For the independent diseases or no co-infections cases, $T^*(S)$ is strictly concave increasing in each π_i, $\forall i \in S$, as long as $\pi(S) \leq \underline{p}$.*

Proof. For both the independent diseases and no co-infections cases, $\pi(S)$ is linear increasing in π_i, $\forall i \in S$ (Eqs. (15)-(16)), and $T^*(\pi(S))$ is strictly concave increasing in $\pi(S) \in [0, \underline{p}]$ (Property 2). Then, it follows that $T^*(\pi(S))$ is strictly concave increasing in $\pi_i, \forall i \in S$, in the region $0 \leq \pi(S) \leq \underline{p}$ [14]. □

Remark A.1. *If $\boldsymbol{\Pi}$ has an interval type uncertainty set given by,*

$$\Omega(\boldsymbol{\Pi}) = \{\boldsymbol{\pi} : \pi_i \in [\underline{\pi}_i, \overline{\pi}_i], i \in N\}, \quad \text{where } 0 \leq \underline{\pi}_i \leq \overline{\pi}_i \leq 1,\ i \in N, \tag{21}$$

then $\boldsymbol{P}$ has the following uncertainty set,

$$\Omega(\boldsymbol{P}) = \{\boldsymbol{p} \geq \boldsymbol{0} : (\boldsymbol{p}; \boldsymbol{\pi}) \text{ is a solution to (5)-(6)}, \quad \boldsymbol{\pi} \in \Omega(\boldsymbol{\Pi})\}, \tag{22}$$

where $(\boldsymbol{p}; \boldsymbol{\pi})$ (i.e., $\boldsymbol{p}$ conditional on $\boldsymbol{\pi}$) represents a solution to (5)-(6) for a given realization $\boldsymbol{\pi} \in \Omega(\boldsymbol{\Pi})$, and set $\Omega(\boldsymbol{P})$ contains all such non-negative solutions, $(\boldsymbol{p}; \boldsymbol{\pi})$, for $\boldsymbol{\pi} \in \Omega(\boldsymbol{\Pi})$. Note that for each given $\boldsymbol{\pi} \in \Omega(\boldsymbol{\Pi})$, there may be multiple solutions, $(\boldsymbol{p}; \boldsymbol{\pi})$, i.e., with different mono-/co-infection probabilities, because the linear system in (5)-(6), where $\boldsymbol{\pi}$ is given, has 2^n unknowns and $n+1$ constraints, where $2^n > n+1, \forall n \geq 2$.
Similarly, given an uncertainty set on $\boldsymbol{P}$, denoted by $\Omega(\boldsymbol{P})$, the uncertainty set on $\boldsymbol{\Pi}$ is given by,

$$\Omega(\boldsymbol{\Pi}) = \{\boldsymbol{\pi} \geq \boldsymbol{0} : (\pi_i; \boldsymbol{p}), i \in N, \text{ is the unique solution to (5)}, \quad \boldsymbol{p} \in \Omega(\boldsymbol{P})\}.$$

Letting $\underline{\pi}_i \equiv \min\{\pi_i \in \Omega(\boldsymbol{\Pi})\}$ and $\overline{\pi}_i \equiv \max\{\pi_i \in \Omega(\boldsymbol{\Pi})\}, \forall i \in N$, we can equivalently write:

$$\Omega(\boldsymbol{\Pi}) = \{\boldsymbol{\pi} : \pi_i \in [\underline{\pi}_i, \overline{\pi}_i], i \in N\}.$$

Thus, given an uncertainty set $\Omega(\boldsymbol{\Pi})$, one can obtain an uncertainty set $\Omega(\boldsymbol{P})$, and vice versa.

A.3 Supporting Results

Theorem A.1. *Consider $\Omega(\boldsymbol{\Pi}) = \{\boldsymbol{\pi} : \pi_i \in [\underline{\pi}_i, \overline{\pi}_i], i \in N\}$, where $0 \leq \underline{\pi}_i \leq \overline{\pi}_i \leq 1,\ i \in N$, and its equivalent $\Omega(\boldsymbol{P})$ from Remark A.1. For any given $\boldsymbol{x}$ and $\boldsymbol{t}$, the inner maximization in* **R-TD** *objective function (14) can be equivalently expressed as follows:*

$$\max_{\boldsymbol{p} \in \Omega(\boldsymbol{P})} \left\{ \lambda \sum_{k=1}^{n} C(\boldsymbol{x}^k, t^k; \boldsymbol{p}) + (1-\lambda) \sum_{k=1}^{n} T(\boldsymbol{x}^k, t^k; \boldsymbol{p}) \right\} = \sum_{k=1}^{n} \left[\max_{\boldsymbol{p} \in \Omega(\boldsymbol{P})} \left\{ \lambda \times C(\boldsymbol{x}^k, t^k; \boldsymbol{p}) + (1-\lambda) \times T(\boldsymbol{x}^k, t^k; \boldsymbol{p}) \right\} \right]$$

$$= \sum_{k=1}^{n} \tilde{c}\left(\sum_{i \in N} x_i^k, \lambda \right) \times \min \left\{ 1,\ \frac{1}{t^k} + 1 - \left(1 - \min \left\{ 1, \sum_{i \in N} \overline{\pi}_i x_i^k \right\} \right)^{t^k} \right\}, \tag{23}$$

where, for each $\boldsymbol{x}$, $\exists \boldsymbol{p} \in \Omega(\boldsymbol{P})$: $\pi(\boldsymbol{x}^k; \boldsymbol{p}) = \min\left\{1, \sum_{i \in N} \overline{\pi}_i x_i^k\right\}$, $\forall k = 1, \cdots, n$.

Corollary A.1. *For the special case where $\sum_{i \in N} \overline{\pi}_i \leq 1$, the worst-case solution, $\pi(\boldsymbol{x}^k; \boldsymbol{p}) = \min\left\{1, \sum_{i \in N} \overline{\pi}_i x_i^k\right\}$, $\forall k = 1, \cdots, n$, is attained at $\boldsymbol{p}$: $p_i = \overline{\pi}_i, \forall i \in N,\ p_{ij} = 0, \forall ij \in N(2),\ p_{ijr} = 0, \forall ijr \in N(3), \cdots, p_{12\ldots n} = 0,\ p_0 = 1 - \sum_{i \in N} \overline{\pi}_i$, that is, each marginal prevalence is at its upper limit and there are no co-infections.*

A.4 Proofs

Proof of Theorem A.1. From the definitions of $\Omega(\mathbf{\Pi})$ and $\Omega(\boldsymbol{P})$ (Eqs. (21), (22)), for any given assay portfolio $\boldsymbol{x} = (\boldsymbol{x}^k)_{k=1,\cdots,n}$ and pool size vector $\boldsymbol{t} = (t^k)_{k=1,\cdots,n}$, we can write:

$$\max_{\boldsymbol{p}\in\Omega(\boldsymbol{P})} \left\{ \lambda \sum_{k=1}^{n} C(\boldsymbol{x}^k, t^k; \boldsymbol{p}) + (1-\lambda) \sum_{k=1}^{n} T(\boldsymbol{x}^k, t^k; \boldsymbol{p}) \right\} \tag{24}$$

$$= \max_{\boldsymbol{p}\in\Omega(\boldsymbol{P})} \left\{ \sum_{k=1}^{n} \tilde{c}\Big(\sum_{i\in N} x_i^k, \lambda \Big) \times \min\left\{ 1,\ \frac{1}{t^k} + 1 - \Big(1 - \pi(\boldsymbol{x}^k; \boldsymbol{p})\Big)^{t^k} \right\} \right\} \tag{25}$$

$$\leq \sum_{k=1}^{n} \left[\max_{\boldsymbol{p}\in\Omega(\boldsymbol{P})} \left\{ \tilde{c}\Big(\sum_{i\in N} x_i^k, \lambda \Big) \times \min\left\{ 1,\ \frac{1}{t^k} + 1 - \Big(1 - \pi(\boldsymbol{x}^k; \boldsymbol{p})\Big)^{t^k} \right\} \right\} \right] \tag{26}$$

$$= \sum_{k=1}^{n} \tilde{c}\Big(\sum_{i\in N} x_i^k, \lambda \Big) \times \max_{\boldsymbol{p}\in\Omega(\boldsymbol{P})} \left\{ \min\left\{ 1,\ \frac{1}{t^k} + 1 - \Big(1 - \pi(\boldsymbol{x}^k; \boldsymbol{p})\Big)^{t^k} \right\} \right\} \tag{27}$$

$$\leq \sum_{k=1}^{n} \tilde{c}\Big(\sum_{i\in N} x_i^k, \lambda \Big) \times \min\left\{ 1,\ \frac{1}{t^k} + 1 - \left(1 - \min\left\{1, \sum_{i\in N} \overline{\pi}_i x_i^k \right\}\right)^{t^k} \right\}, \tag{28}$$

where (25) follows by Eqs. (2) and (8); (26) follows because, for a given assay portfolio $\boldsymbol{x} = (\boldsymbol{x}^k)_{k=1,\cdots,n}$, and pool size vector $\boldsymbol{t} = (t^k)_{k=1,\cdots,n}$, the solution to (24) requires a *common* worst-case vector, $\boldsymbol{p} \in \Omega(\boldsymbol{P})$, for *all* assays $k = 1, \cdots, n$, while (26) relaxes this restriction and allows for a potentially different worst-case vector, $\boldsymbol{p}^k \in \Omega(\boldsymbol{P})$, for *each* assay $k = 1, \cdots, n$. (27) follows because the term, $\sum_{k=1}^{n} \tilde{c}\Big(\sum_{i\in N} x_i^k, \lambda \Big)$, is a constant for a given $\boldsymbol{x}$, that is, independent of the joint vector $\boldsymbol{p}$. Finally, the upper bound in (28) follows, because for any given $\boldsymbol{x}^k$ and t^k, the term, $\frac{1}{t^k} + 1 - \big(1 - \pi(\boldsymbol{x}^k; \boldsymbol{p})\big)^{t^k}$ is strictly increasing in $\pi(\boldsymbol{x}^k; \boldsymbol{p})$, and $\pi(\boldsymbol{x}^k; \boldsymbol{p}) \leq \min\left\{1, \sum_{i\in N} \overline{\pi}_i x_i^k\right\}, \forall k = 1, \cdots, n$.

Then, to prove the equivalence of (24) and (27), and the equivalence of (27) and (28), it is sufficient to show that, for a given $\boldsymbol{x}$ and $\boldsymbol{t}$, there exists a *common* $\boldsymbol{p} \in \Omega(\boldsymbol{P})$ (for all assays $1, \cdots, n$) that corresponds to the upper bound in (28), in which case this particular $\boldsymbol{p}$ must be a worst-case solution to both (24) and (27). Equivalently, we wish to show that $\exists \boldsymbol{p} \geq \boldsymbol{0}$ that satisfies: **(i) (assay equations)** $\pi(\boldsymbol{x}^k; \boldsymbol{p}) = \min\left\{1, \sum_{i\in N} \overline{\pi}_i x_i^k\right\}, \forall k = 1, \cdots, n$; **(ii) (disease equations)** $Pr(A_i^+; \boldsymbol{p}) = \overline{\pi}_i$; and **(iii) (universal set equation)** $(\pi(N); \boldsymbol{p}) + p_0 = 1$, where (i) follows from (28), and (ii)-(iii), along with the constraint $\boldsymbol{p} \geq \boldsymbol{0}$, ensure that $\boldsymbol{p} \in \Omega(\boldsymbol{P})$.

Observe that if an assay has exactly one disease, then its assay equation reduces to the equation for its corresponding disease, and is redundant; these assay equations are omitted from further consideration. Therefore, we only consider the assay equations for assays in $Q \equiv \{k = 1, \cdots, n : s^k \geq 2\}$, with cardinality $m : 0 \leq m \leq \lfloor \frac{n}{2} \rfloor$; without loss of generality, the assays in Q are re-indexed as assays $1, \cdots, m$. Thus, we wish to show that the following system of linear equations (based on Eqs. (3)-(5)) has a non-negative solution, $\boldsymbol{p} \in \Re^{+(2^n)\times 1}$:

$$p_i + \textstyle\sum_{j:ij\in N(2)} p_{ij} + \sum_{j:ji\in N(2)} p_{ji} + \cdots + p_{12\cdots n} = \overline{\pi}_i, \quad \forall i \in N, \tag{29}$$

$$\textstyle\sum_{i\in S^k} p_i + \sum_{ij\in N(2): i\in S^k} \text{or } {}_{j\in S^k} p_{ij} + \cdots + p_{12\ldots n} = \min\left\{1, \sum_{i\in S^k} \overline{\pi}_i\right\}, \quad \forall k \in Q \tag{30}$$

$$p_0 + \textstyle\sum_{i\in N} p_i + \sum_{ij\in N(2)} p_{ij} + \sum_{ijr\in N(3)} p_{ijr} \ + \cdots + p_{12\ldots n} = 1. \tag{31}$$

This system has $n + m + 1$ linearly independent equations and 2^n unknowns (i.e., each element of $\boldsymbol{p}$), where $2^n \geq n + \lfloor \frac{n}{2} \rfloor + 1, \forall n \geq 2$. Let $\boldsymbol{A} \in \{0,1\}^{(n+m+1)\times(2^n)}$ and $\boldsymbol{b} \in \Re^{+(n+m+1)\times 1}$ respectively denote the binary coefficient matrix, and the non-negative RHS of this system of linear equations. By Farka's Lemma [29], exactly one of the following alternatives hold:

1. Either $\exists \boldsymbol{p} \in \Re^{(2^n)\times 1}$ that satisfies:

$$\boldsymbol{A}\boldsymbol{p} = \boldsymbol{b}, \quad \boldsymbol{p} \geq \boldsymbol{0}, \tag{F-1}$$

2. or else $\exists \boldsymbol{y} = (u_1, \cdots, u_n, v_1, \cdots, v_m, z) \in \Re^{(n+m+1)\times 1}$, where u_i corresponds to (29) for disease $i \in N$, v_k corresponds to (30) for assay $k \in Q$, and z corresponds to (31), that satisfies:

$$\boldsymbol{A}^T\boldsymbol{y} \geq \boldsymbol{0}, \tag{F-2a}$$

$$\boldsymbol{b}^T\boldsymbol{y} < 0. \tag{F-2b}$$

Then it suffices to show that $\nexists \boldsymbol{y} \in \Re^{(n+m+1)\times 1}$ that satisfies (F-2a)-(F-2b), which we prove by contradiction.

To simplify the subsequent presentation, we introduce some new notation. Denote the columns of $\boldsymbol{A}$ by $\boldsymbol{A}_x, x = 1, \cdots, 2^n$, where each column represents the constraint coefficients for an element of $\boldsymbol{p} \in \Re^{+(2^n)\times 1}$. Hence, each row of (F-2a), $\boldsymbol{A}_x^T\boldsymbol{y} \geq 0$, corresponds to an element of $\boldsymbol{p}$, which is the probability of either a mono-infection ($p_i, i \in N$), a co-infection ($p_{i\cdots j}, i, \cdots, j \in N$), or no-infection ($p_0$); in either case, it is the probability of a set of diseases $S \subseteq N$ (e.g., $S = \{i\}, S = \{i, \cdots, j\}, S = \emptyset$), and we refer to this inequality as (F-2a)(S), i.e., indexed by its disease set. As defined above, $\boldsymbol{p}, \boldsymbol{b}$, and $\boldsymbol{y}$ are all column vectors.

Suppose, to the contrary, that $\boldsymbol{y} = (u_1, \cdots, u_n, v_1, \cdots, v_m, z)$ is a solution to (F-2a)-(F-2b). Based on the signs of assay and disease variables, we decompose assay set Q into six mutually exclusive sets:

$$\begin{aligned}
&Q_{a+}^{i+} \equiv \{k \in Q : v_k \geq 0 \text{ and } \{u_i \geq 0, \forall i \in S^k\}\}, \quad Q_{a+}^{i-} \equiv \{k \in Q : v_k \geq 0 \text{ and } \{u_i < 0, \forall i \in S^k\}\},\\
&Q_{a+}^{i0} \equiv \{k \in Q : v_k \geq 0 \text{ and } \{\exists i \in S^k : u_i \geq 0, \text{ and } \exists j \in S^k : u_j < 0\}\},\\
&Q_{a-}^{i+} \equiv \{k \in Q : v_k < 0 \text{ and } \{u_i \geq 0, \forall i \in S^k\}\}, \quad Q_{a-}^{i-} \equiv \{k \in Q : v_k < 0 \text{ and } \{u_i < 0, \forall i \in S^k\}\},\\
&Q_{a+}^{i0} \equiv \{k \in Q : v_k < 0 \text{ and } \{\exists i \in S^k : u_i \geq 0, \text{ and } \exists j \in S^k : u_j < 0\}\}.
\end{aligned} \tag{32}$$

Observe that we use the indices k and i to respectively refer to assay variables and disease variables; the subscripts $a+$ and $a-$ to respectively refer to assay sets with non-negative versus negative assay variables (v_k); and the superscripts $i+$, $i-$, and $i0$ to respectively refer to assay sets whose corresponding disease variables (u_i) are all non-negative, negative, or neither. Finally, let $S^{k-} \equiv \{i \in S^k : u_i < 0\}, k \in Q$.

The proof by contradiction proceeds in two parts. First we consider a specific row of (F-2a) and perform row operations on it in order to bring it into a form that is a lower bound for the LHS of (F-2b), and show that this lower bound is non-negative, thus reaching a contradiction with the inequality in (F-2b).

To this end, consider disease set $\tilde{S} \subseteq N$:

$$\tilde{S} \equiv \bigcup_{k \in Q_{a-}^{i+}} argmin_{i \in S^k}\{u_i\} \bigcup_{k \in Q_{a+}^{i-} \cup Q_{a-}^{i-}} S^k \bigcup_{k \in Q_{a+}^{i0} \cup Q_{a-}^{i0}} S^{k-},$$

which contains the disease with the lowest u_i value (breaking ties arbitrarily) for each assay $k \in Q_{a-}^{i+}$, all the diseases in assays $k \in Q_{a+}^{i-} \cup Q_{a-}^{i-}$, and all the diseases with a negative u_i value in assays $k \in Q_{a+}^{i0} \cup Q_{a-}^{i0}$. (As will be clear in the sequel, the diseases in the assays in set Q_{a+}^{i+} are omitted, as their assay and disease variables are non-negative by definition). Consider the joint (co-infection) probability of the $\tilde{s}$ diseases in set $\tilde{S}$, and its corresponding column $\boldsymbol{A}_x$, where $\boldsymbol{A}_{i,x} = 1$, $\forall i \in \tilde{S}$, $\boldsymbol{A}_{n+k,x} = 1$ if $\tilde{S} \cap S^k \neq \emptyset$, $\forall k \in Q$, $\boldsymbol{A}_{n+m+1,x} = 1$, and $\boldsymbol{A}_{j,x} = 0$ otherwise. Then:

$$\text{(F-2a)}(\tilde{S}): \quad z + \sum_{k \in Q_{a+}^{i-}} v_k + \sum_{k \in Q_{a+}^{i0}} v_k + \sum_{k \in Q_{a-}^{i+}} v_k + \sum_{k \in Q_{a-}^{i-}} v_k + \sum_{k \in Q_{a-}^{i0}} v_k + \sum_{k \in Q_{a+}^{i-}} \sum_{i \in S^k} u_i + \sum_{k \in Q_{a+}^{i0}} \sum_{i \in S^{k-}} u_i$$

$$+ \sum_{k\in Q^{i+}_{a-}} \min_{i\in S^k}\{u_i\} + \sum_{k\in Q^{i-}_{a-}} \sum_{i\in S^k} u_i + \sum_{k\in Q^{i0}_{a-}} \sum_{i\in S^{k-}} u_i \geq 0.$$

Through a series of row operations (detailed below), (F-2a)($\tilde{S}$) can be converted into the following form:

$$\begin{aligned} z + \sum_{k\in Q^{i-}_{a+}} v_k \times \max_{i\in S^k}\{\overline{\pi}_i\} + \sum_{k\in Q^{i0}_{a+}} v_k \times \max_{i\in S^{k-}}\{\overline{\pi}_i\} + \sum_{k\in Q^{i+}_{a-}\cup Q^{i-}_{a-}\cup Q^{i0}_{a-}} v_k \times \min\{1, \sum_{i\in S^k}\overline{\pi}_i\} \\ + \sum_{k\in Q^{i+}_{a-}} \min_{i\in S^k}\{u_i\} \times \min\{1, \sum_{j\in S^k}\overline{\pi}_j\} + \sum_{k\in Q^{i-}_{a+}} \sum_{i\in S^k} u_i \times \max_{j\in S^k}\{\overline{\pi}_j\} + \sum_{k\in Q^{i-}_{a-}} \sum_{i\in S^k} u_i \times \min\{1, \sum_{j\in S^k}\overline{\pi}_j\} \\ + \sum_{k\in Q^{i0}_{a+}} \sum_{i\in S^{k-}} u_i \times \max_{j\in S^{k-}}\{\overline{\pi}_j\} + \sum_{k\in Q^{i0}_{a-}} \sum_{i\in S^{k-}} u_i \times \min\{1, \sum_{j\in S^k}\overline{\pi}_j\} \geq 0. \end{aligned} \tag{33}$$

In the remainder of the proof, we first describe the bounding of the LHS of (33), so as to reach a contradiction based on Farka's Lemma. Then we detail the specific row operations needed to convert (F-2a)($\tilde{S}$) to (33).

Bounding the LHS of (33): We bound the LHS of (33) to reach the form in (F-2b), i.e., $\boldsymbol{b}^T\boldsymbol{y}$, where each $v_k, k \in Q$, has a coefficient of $\min\{1, \sum_{i\in S^k}\overline{\pi}_i\}$, each $u_i, i \in N$, has a coefficient of $\overline{\pi}_i$, and z has a coefficient of 1. We do this through a series of upper bounds on the different terms in (33). First, because $0 \leq \overline{\pi}_i \leq 1, \forall i \in N$:

$$\overline{\pi}_j \leq \max_{i\in S}\{\overline{\pi}_i\} \leq \min\{1, \sum_{i\in S}\overline{\pi}_i\}, \quad \forall j \in S,\ S \subseteq N. \tag{34}$$

The next set of inequalities follow by (34), the definitions in (32), the definition of S^{k-}, and because $S^{k-} \subseteq S^k, \forall k \in Q$:

$$\sum_{k\in Q^{i+}_{a+}} v_k \times \min\{1, \sum_{i\in S^k}\overline{\pi}_i\} + \sum_{k\in Q^{i+}_{a+}} \sum_{i\in S^k} u_i\overline{\pi}_i \geq 0 \tag{35}$$

$$\sum_{k\in Q^{i-}_{a+}} v_k \times \max_{i\in S^k}\{\overline{\pi}_i\} \leq \sum_{k\in Q^{i-}_{a+}} v_k \times \min\{1, \sum_{i\in S^k}\overline{\pi}_i\} \tag{36}$$

$$\sum_{k\in Q^{i0}_{a+}} v_k \times \max_{i\in S^{k-}}\{\overline{\pi}_i\} \leq \sum_{k\in Q^{i0}_{a+}} v_k \times \max_{i\in S^k}\{\overline{\pi}_i\} \leq \sum_{k\in Q^{i0}_{a+}} v_k \times \min\{1, \sum_{i\in S^k}\overline{\pi}_i\} \tag{37}$$

$$\sum_{k\in Q^{i+}_{a-}} \min_{i\in S^k}\{u_i\} \times \min\{1, \sum_{j\in S^k}\overline{\pi}_j\} \leq \sum_{k\in Q^{i+}_{a-}} \min_{i\in S^k}\{u_i\} \times \sum_{j\in S^k}\overline{\pi}_j \leq \sum_{k\in Q^{i+}_{a-}} \sum_{i\in S^k} u_i\overline{\pi}_i \tag{38}$$

$$\sum_{k\in Q^{i-}_{a+}} \sum_{i\in S^k} u_i \times \max_{j\in S^k}\{\overline{\pi}_j\} \leq \sum_{k\in Q^{i-}_{a+}} \sum_{i\in S^k} u_i\overline{\pi}_i \tag{39}$$

$$\sum_{k\in Q^{i-}_{a-}} \sum_{i\in S^k} u_i \times \min\{1, \sum_{j\in S^k}\overline{\pi}_j\} \leq \sum_{k\in Q^{i-}_{a-}} \sum_{i\in S^k} u_i\overline{\pi}_i \tag{40}$$

$$\sum_{k\in Q^{i0}_{a+}} \sum_{i\in S^{k-}} u_i \times \max_{j\in S^{k-}}\{\overline{\pi}_j\} \leq \sum_{k\in Q^{i0}_{a+}} \sum_{i\in S^{k-}} u_i\overline{\pi}_i \leq \sum_{k\in Q^{i0}_{a+}} \sum_{i\in S^k} u_i\overline{\pi}_i \tag{41}$$

$$\sum_{k\in Q^{i0}_{a-}} \sum_{i\in S^{k-}} u_i \times \min\{1, \sum_{j\in S^k}\overline{\pi}_j\} \leq \sum_{k\in Q^{i0}_{a-}} \sum_{i\in S^{k-}} u_i\overline{\pi}_i \leq \sum_{k\in Q^{i0}_{a-}} \sum_{i\in S^k} u_i\overline{\pi}_i. \tag{42}$$

Using (35)-(42), we can bound the LHS of (33) as follows:

$$\text{RHS of (33)} = 0 \leq \text{ LHS of (33)} \ \leq z + \sum_{k\in Q} v_k \times \min\{1, \sum_{i\in S^k}\overline{\pi}_i\} + \sum_{i\in N} u_i\overline{\pi}_i = \boldsymbol{b}^T\boldsymbol{y}, \tag{43}$$

which contradicts with (F-2a):

$$\text{(F-2a)}: \ \boldsymbol{b}^T\boldsymbol{y} < 0 \Leftrightarrow z + \sum_{k\in Q} v_k \times \min\{1, \sum_{i\in S^k}\overline{\pi}_i\} + \sum_{i\in N} u_i\overline{\pi}_i < 0,$$

that is, $\nexists \boldsymbol{y} \in \Re^{(n+m+1)\times 1}$ that satisfies (F-2a) and (F-2b). Then, by Farka's Lemma, system (F-1) must have a

solution, that is, $\exists \boldsymbol{p} \in \Re^{(2^n)\times 1}$ such that $\boldsymbol{Ap} = \boldsymbol{b}$ and $\boldsymbol{p} \geq \boldsymbol{0}$. Then to complete the proof, it is sufficient to detail the row operations needed to convert (F-2a)$(\tilde{S})$ to (33), which we do next.

Row operations: We define the *assay multipliers*, $\Delta_k, k \in Q \setminus Q^{i+}_{a+}$, as:

$$\Delta_k = \begin{cases} \sum_{i\in S^k} \overline{\pi}_i, & \forall k \in Q^{i+}_{a-} \cup Q^{i-}_{a-} \cup Q^{i0}_{a-} \\ \max_{i\in S^k}\{\overline{\pi}_i\}, & \forall k \in Q^{i-}_{a+} \\ \max_{i\in S^{k-}}\{\overline{\pi}_i\}, & \forall k \in Q^{i0}_{a+} \end{cases}, \tag{44}$$

and construct the **ordered vector $\boldsymbol{\Delta}$** to include any $\Delta_k < 1, k \in Q \setminus Q^{i+}_{a+}$, and the element 1, which are re-indexed following a non-decreasing order, i.e., $\Delta_1 \leq \Delta_2 \leq \cdots \leq \Delta_H < 1 = \Delta_{H+1}$, that is, $\boldsymbol{\Delta}$ includes the multipliers for all assays in $Q^{i-}_{a+} \cup Q^{i0}_{a+}$; and the multipliers for assays in $Q^{i+}_{a-} \cup Q^{i-}_{a-} \cup Q^{i0}_{a-}$ *only if* they are strictly less than 1. We use index h to refer to the elements of $\boldsymbol{\Delta}$. By construction, each $\Delta_h, h = 1, \cdots, H$, is the multiplier of at least one assay, denoted by assays $k_h, \cdots, l_h$, and $\Delta_{H+1} = 1$.

In what follows, we will conduct a series of row operations on (F-2a)$(\tilde{S})$, i.e., the inequality for the joint probability of the diseases in set $\tilde{S}$. In each row operation, we will modify set $\tilde{S}$ by progressively removing a certain subset of diseases. Then we will consider the inequality in (F-2a) for the joint probability of the *remaining* diseases in this set, multiply both sides of this inequality by certain assay multipliers, and add to (F-2a)$(\tilde{S})$, until we reach the desired form, (33). In the following, we describe this series of row operations first in words, then using mathematical notation.

We first multiply both sides of (F-2a)$(\tilde{S})$ by Δ_1, i.e., the assay multiplier for assays $k_1, \cdots, l_1$; multiply (F-2a)$(\tilde{S} \setminus \cup_{k_1,\cdots,l_1} S^k)$ by $\Delta_2 - \Delta_1$; and add the two inequalities to obtain a new inequality (F-2a)$(\tilde{S})_{(1)}$ in which the variables for assays $k_1, \cdots, l_1$ and their diseases ($v_k, k = k_1, \cdots, l_1$, and $u_i, i \in \cup_{k=k_1,\cdots,l_1} S^k$) are multiplied by Δ_1, and all the remaining variables (in $\boldsymbol{y}$) are multiplied by Δ_2. Next, we consider Δ_2, i.e., the multiplier for assays $k_2, \cdots, l_2$; multiply (F-2a)$(\tilde{S} \setminus \cup_{k_1,\cdots,l_1,k_2,\cdots,l_2} S^k)$ by $(\Delta_3 - \Delta_2)$; and add to (F-2a)$(\tilde{S})_{(1)}$, to obtain a new inequality in which the variables for assays $k_1, \cdots, l_1$ and their diseases are multiplied by Δ_1, for assays $k_2, \cdots, l_2$ and their diseases are multiplied by Δ_2, and the remaining variables are multiplied by Δ_3. We repeat this process until H such row operations are completed, at which point we reach (33). In the following, we formally describe this series of row operations. To this end, at each iteration h, that is, when considering $\Delta_h, h = 1, \cdots, H$, we update the assay and disease sets as follows:

$$Q^{i+}_{a-}(\Delta_h) \equiv \left\{ k \in Q^{i+}_{a-} : \sum_{i\in S^k} \overline{\pi}_i > \Delta_h \right\},\ Q^{i-}_{a+}(\Delta_h) \equiv \left\{ k \in Q^{i-}_{a+} : \max_{i\in S^k}\{\overline{\pi}_i\} > \Delta_h \right\},\ Q^{i0}_{a+}(\Delta_h) \equiv \left\{ k \in Q^{i0}_{a+} : \max_{i\in S^{k-}}\{\overline{\pi}_i\} > \Delta_h \right\},$$

$$Q^{i-}_{a-}(\Delta_h) \equiv \left\{ k \in Q^{i-}_{a-} : \sum_{i\in S^k} \overline{\pi}_i > \Delta_h \right\},\ Q^{i0}_{a-}(\Delta_h) \equiv \left\{ k \in Q^{i0}_{a-} : \sum_{i\in S^k} \overline{\pi}_i > \Delta_h \right\}, \text{ and}$$

$$\tilde{S}(\Delta_h) \equiv \bigcup_{k\in Q^{i+}_{a-}(\Delta_h)} argmin_{i\in S^k}\{u_i\} \bigcup_{k\in Q^{i-}_{a+}(\Delta_h)\cup Q^{i-}_{a-}(\Delta_h)} S^k \bigcup_{k\in Q^{i0}_{a+}(\Delta_h)\cup Q^{i0}_{a-}(\Delta_h)} S^{k-},$$

that is, when considering Δ_h, we remove the diseases of assays $k_h, \cdots, l_h$ from the previous disease set $\tilde{S}(\Delta_{h-1})$. The row operations follow:

1. $\Delta_1 \times$(F-2a)$(\tilde{S})$ $+(\Delta_2 - \Delta_1) \times$(F-2a)$(\tilde{S}(\Delta_1)) \equiv$ (F-2a)$(\tilde{S})_{(1)}$: In the new inequality (F-2a)$(\tilde{S})_{(1)}$, the variables for assays $k_1, \cdots, l_1$ and their diseases ($v_k, k = k_1, \cdots, l_1$, and $u_i, i \in \cup_{k=k_1,\cdots,l_1} S^k$) are multiplied by Δ_1, and all the remaining variables (in $\boldsymbol{y}$) are multiplied by Δ_2.
2. For $h = 2, \cdots, H$: (F-2a)$(\tilde{S})_{(h-1)}$ $+ (\Delta_{h+1} - \Delta_h) \times$ (F-2a)$(\tilde{S}(\Delta_h)) \equiv$ (F-2a)$(\tilde{S})_{(h)}$: In the new inequality (F-2a)$(\tilde{S})_{(h)}$, the variables for assays $k_1, \cdots, l_1$ and their diseases are multiplied by Δ_1, for assays $k_2, \cdots, l_2$ and their diseases are multiplied by Δ_2, and so on, and for assays $k_h, \cdots, l_h$ and their diseases are multiplied by Δ_h, and all the remaining variables are multiplied by Δ_{h+1}.

3. At the conclusion of row operation H, the final inequality (F-2a)$(\tilde{S})_{(H)}$ is such that the variables for assays $k_1, \cdots, l_1$ and their diseases are multiplied by Δ_1, for assays $k_2, \cdots, l_2$ and their diseases are multiplied by Δ_2, for assays $k_3, \cdots, l_3$ and their diseases are multiplied by Δ_3, and so on, for assays $k_H, \cdots, l_H$ and their diseases multiplied by Δ_H, and all the remaining variables are multiplied by 1, that is, we reach (33). This completes the proof. □

Proof of Corollary A.1. The result follows directly from Theorem A.1 because $\pi(\boldsymbol{x}^k;\boldsymbol{p}) = \sum_{i\in N}\overline{\pi}_i x_i^k \leq \pi(N) = \sum_{i\in N}\overline{\pi}_i \leq 1,\ \forall k = 1,\cdots,n$. Hence, to conserve $\pi(\boldsymbol{x}^k;\boldsymbol{p}) = \sum_{i\in N}\overline{\pi}_i x_i^k,\ \forall k = 1,\cdots,n$, and $\pi(N) = \sum_{i\in N}\overline{\pi}_i$, we have that $p_i = \overline{\pi}_i, \forall i \in N,\ p_{ij} = 0, \forall ij \in N(2),\ p_{ijr} = 0, \forall ijr \in N(3), \cdots, p_{12\ldots n} = 0,\ p_0 = 1 - \sum_{i\in N}\overline{\pi}_i$. □

Proof of Property 2. Part 1. For any assay $S \subseteq N$, from Eq. (8):

$$T(S,t) = \frac{1}{t} + 1 - (1-\pi(S))^t \leq T(S,1) = 1 \Leftrightarrow \pi(S) \leq 1 - \sqrt[t]{\frac{1}{t}}, \qquad \text{where } \frac{\partial\left(1-\sqrt[t]{\frac{1}{t}}\right)}{\partial t} = -t^{\left(\frac{-2t-1}{t}\right)}\left[ln(t)-1\right] > 0 \Leftrightarrow t < e,$$

that is, function $1 - \sqrt[t]{\frac{1}{t}}$ is strictly increasing for $t \leq 2$, and strictly decreasing for $t \geq 3$. Therefore, the maximum value of $\pi(S)$ for which pooled testing, with integer pool sizes, outperforms individual testing is attained at $t = 2$ or $t = 3$. Then, the prevalence threshold, $\underline{p}$, follows because $1 - \sqrt[2]{\frac{1}{2}} = 0.292893 < 1 - \sqrt[3]{\frac{1}{3}} = 0.306639 \approx 0.31$. □

Proof of Theorem 1. The following relationships hold $\forall\lambda \in [0,1]$, hence we drop λ as an argument.

By Property A.1, $\tilde{c}(s)$ is subadditive in s, that is, $\tilde{c}(s) \leq \sum_{k=1}^{q}\tilde{c}(s^k),\ \forall s^k \in Z^+ : \sum_{k=1}^{q} s^k = s, \forall q = 2,\cdots,s, \forall s \in Z^+$ (Definition A1). Then, from Eq. (18), $TC_{I^{(1)}} = \tilde{c}(n) \leq \sum_{k=1}^{q}\tilde{c}(s^k_{I^{(q)}}) = TC_{I^{(q)}},\ \forall q = 2,\cdots,n$, hence $I^{(1)} \preceq I^{(q)},\ \forall q = 2,\cdots,n$. Let $S \subseteq N$ denote the set of diseases that are individually tested in an optimal $M^{(q)}$ design. For set S, $TC_{I^{(1)}}(S) = \tilde{c}(s) \leq \sum_{k=1}^{q}\tilde{c}(s^k_{I^{(q)}}) = TC_{I^{(q)}}(S),\ \forall q = 2,\cdots,s, S \subseteq N$, hence, it is optimal to bundle all the diseases in set S into one multiplex and individually test.

Part 1. Case where $\pi(N) \leq \underline{p}$: Then, $\pi(S) \leq \pi(N) \leq \underline{p}, \forall S \subseteq N \Rightarrow T_D^*(S) \leq T(S,1) = 1, \forall S \subseteq N$ (Property 2). Hence by Eqs. (18)-(19), $TC_{D^{(1)}} = \tilde{c}(n) \times T_D^*(N) \leq \tilde{c}(n) \times T(N,1) = TC_{I^{(1)}}$, hence $D^{(1)} \preceq I^{(1)} \Rightarrow D^{(1)} \preceq I^{(q)}$, $\forall q = 1,\cdots,n$, because $I^{(1)} \preceq I^{(q)},\ \forall q = 2,\cdots,n$, as shown above.

Next, consider an $M^{(q)}$ design, which, by definition and as shown above, must contain exactly one assay with individual testing, say assay $k' \in \{1,\cdots,q\} : T(S^{k'}_{M^{(q)}}, t^{k'}_{M^{(q)}} = 1) = 1$. Construct an alternative design that uses the $M^{(q)}$ partition, $\boldsymbol{S}_{M^{(q)}}$, but with pool size vector $\boldsymbol{t}_D^*(\boldsymbol{S}_{M^{(q)}}) \geq \boldsymbol{2}$, that is, each assay in the $M^{(q)}$ partition is now pooled with the optimal pool size. Because $\pi(S) \leq \underline{p}, \forall S \subseteq N$, we have that $T_D^*(S^k_{M^{(q)}}) \leq T(S^k_{M^{(q)}}, 1)$, $\forall k = 1,\cdots,q$ (Property 2), that is, this new design, where all assays are pooled, is a feasible design for the $D^{(q)}$ class, and has a total cost less than or equal to that of the optimal $M^{(q)}$ design. This inequality continues to hold for the optimal $D^{(q)}$ design, and we have that $D^{(q)} \preceq M^{(q)}, \forall q = 2,\cdots,n$. Therefore, the optimal design class is $D^{(q)}$, for some $q = 1,\cdots,n$.

Part 2. Case where $\pi_1 \leq \underline{p} < \pi(N)$: By Property 2, $1 = T(N,1) \leq T_D^*(N) \Rightarrow I^{(1)} \preceq D^{(1)}$; and from Eq. (7), $\pi_1 \leq \underline{p} \Rightarrow \pi_i \leq \underline{p}, \forall i \in N \Rightarrow T_D^*(\{i\}) \leq T(\{i\},1) = 1,\ \forall i \in N$. Then, because $M^{(n)}$ is an all-singleton design, i.e., $\boldsymbol{S} = (\{i\})_{i\in N}$, it must have exactly one assay that is individually tested, and the total cost of this design can be improved by pooling this particular assay (with optimal pool size), hence converting the design into a feasible $D^{(n)}$ design with a total cost less than or equal to the optimal $M^{(n)}$ design. This inequality continues to hold for the optimal $D^{(n)}$ design, and we have that $D^{(n)} \preceq M^{(n)}$. Therefore, the optimal design class is either $I^{(1)}$, or $D^{(q)}$ for some $q = 2,\cdots,n$, or $M^{(q)}$ for some $q = 2,\cdots,n-1$.

Part 3. Case where $\pi_n \geq \underline{p}$: Then, $\underline{p} \leq \pi_n \leq \pi(S), \forall S \subseteq N \Rightarrow T(S,1) = 1 \leq T_D^*(S), \forall S \subseteq N$ (Property 2). Therefore, we can show that $I^{(q)} \preceq D^{(q)}, \forall q = 1,\cdots,n$, and $I^{(q)} \preceq M^{(q)}, \forall q = 2,\cdots,n$. Further, $I^{(1)} \preceq I^{(q)}, \forall q = 2,\cdots,n$ as shown above. Then, the optimal design class is $I^{(1)}$.

Part 4. Case where $\exists i \in \{1,\cdots,n-1\} : \pi_{i+1} < \underline{p} < \pi_i$: Then, $\pi(N) \geq \pi_1 \geq \pi_2 \cdots \geq \pi_i > \underline{p}$. Hence, by Property 2, $1 = T(N,1) \leq T_D^*(N) \Rightarrow I^{(1)} \preceq D^{(1)}$. Consider any $D^{(q)}$, $q = 2,\cdots,n$, which must contain at least one assay $k' \in \{1,\cdots,q\} : \pi(S_{D^{(q)}}^{k'}) > \underline{p} \Rightarrow T_D^*(S_{D^{(q)}}^{k'}) > T(S_{D^{(q)}}^{k'},1) = 1$ (Property 2). Construct an alternative design that uses the $D^{(q)}$ partition, $\boldsymbol{S}_{D^{(q)}}$, but with $t_{D^{(q)}}^{k'} = 1$, which is a feasible design for the $M^{(q)}$ class, and has a total cost less than or equal to that of the optimal $D^{(q)}$ design. This inequality continues to hold for the optimal $M^{(q)}$ design, and we have that $M^{(q)} \preceq D^{(q)}, \forall q = 2,\cdots,n$.
Next consider set $S = \{1,\cdots i\} \subset N$, where $\pi(S) \geq \pi_i > \underline{p}$. Then, by part 3 of this theorem, $I^{(1)}$ is optimal for set S, that is, in an optimal design, all the diseases in set S are bundled into one multiplex and individually tested. Because the total cost is additive over assay sets; the set of remaining diseases, $N \setminus S = \{i+1,\cdots,n\}$, has cardinality $n-i$; and all the i diseases in set S are bundled into one multiplex assay, an optimal design can have at most $n-i+1$ assays, exactly one of which is individually tested. That is, the optimal design class is either $I^{(1)}$, or $M^{(q)}$ for some $q = 2,\cdots,n-i+1$. □

Proof of Property 3. The first part follows from Definition 5 because $\tilde{c}(s,\lambda) = \lambda\ c(s) + 1 - \lambda$ is a linear function of $c(s), s \in Z^+$. For the second part, for any $\epsilon \in (0,\lambda)$, we have:

$$\tilde{c}(s+1,\lambda-\epsilon) - \tilde{c}(s,\lambda-\epsilon) = (\lambda-\epsilon)\ [c(s+1)-c(s)] \quad \leq \quad \lambda\ [c(s+1)-c(s)] = \tilde{c}(s+1,\lambda) - \tilde{c}(s,\lambda),\ \forall s \in Z^+,$$

and it follows, by Definition 5, that $\tilde{c}(s,\lambda) \geq_{diff} \tilde{c}(s,\lambda-\epsilon)$. □

Proof of Theorem 2. Consider the smallest-difference composite cost function in $\tilde{C}$, that is, $\lambda = 0$ or $c(s) = \gamma, \forall s \in Z^+$, where the objective reduces to the minimization of the expected tests function, $T(.)$. By Theorem 1, $I^{(1)} \preceq I^{(q)}, \forall q = 2,\cdots,n$, and an $M^{(q)}$ design contains exactly one assay with individual testing, that is, $T(S_{M^{(q)}}^{k'}, t_{M^{(q)}}^{k'} = 1) = 1$, for some $k' \in \{1,\cdots,q\}$, and $q-1$ assays are pooled. Then, $T_{M^{(q)}} > 1 = T_{I^{(1)}}$, hence $I^{(1)} \preceq M^{(q)}, \forall q = 2,\cdots,n$. Next we characterize an optimal design class.

Part 1. Case where $\pi(N) \leq \underline{p}$: By Theorem 1, the optimal design class is $D^{(q)}$, for some $q = 1,\cdots,n$. Consider a $D^{(q)}$ design, with partition $\boldsymbol{S}_{D^{(q)}}$, for any $q = 2,\cdots,n$. We construct a feasible $D^{(q-1)}$ design by combining any two sets, $S_{D^{(q)}}^{k_1}$ and $S_{D^{(q)}}^{k_2}$, $k_1, k_2 \in \{1,\cdots,q\}, k_1 \neq k_2$, into a new set, $S^k \equiv S_{D^{(q)}}^{k_1} \cup S_{D^{(q)}}^{k_2}$. Because $T_D^*(S)$ is subadditive in $\pi(S) \in [0,\underline{p}]$, and $\pi(S)$ is subadditive in set S (Property A.1), it follows that $\sum_{r=1}^{2} T_D^*(S^{k_r}) \geq T_D^*(S^k)$. Because this result holds for a feasible $D^{(q-1)}$ design, it continues to hold for an optimal $D^{(q-1)}$ design, hence $D^{(q-1)} \preceq D^{(q)}$, for all $q = 2,\cdots,n$. Consequently, $D^{(1)} \preceq D^{(2)} \preceq \cdots \preceq D^{(n)}$, and the optimal design class is $D^{(1)}$.

Part 2. Case where $\pi(N) > \underline{p}$: Because $I^{(1)} \preceq I^{(q)}$ and $I^{(1)} \preceq M^{(q)}, \forall q = 2,\cdots,n$, it is sufficient to show that $I^{(1)} \preceq D^{(q)}$, that is, $T_{I^{(1)}} \leq T_{D^{(q)}}, \forall q = 1,\cdots,n$. By Property 2, $1 = T_{I^{(1)}} < T_D^*(N) = T_{D^{(1)}}$, hence $I^{(1)} \preceq D^{(1)}$. Next consider any Dorfman design, $D^{(q)}, q = 2,\cdots,n$, with partition $\boldsymbol{S}_{D^{(q)}}$. There are two possible cases:

(a) Case where $\exists k' \in \{1,\cdots,q\} : \pi(S_{D^{(q)}}^{k'}) > \underline{p}$: Then, we have that $T_D^*(S_{D^{(q)}}^{k'}) > T(S_{D^{(q)}}^{k'},1) = 1$ (Property 2), and it trivially follows that $1 = T_{I^{(1)}} < \sum_{k=1}^{q} T_D^*(S_{D^{(q)}}^k)$, hence $I^{(1)} \preceq D^{(q)}, \forall q = 2,\cdots,n$.

(b) Case where $\pi(S_{D^{(q)}}^k) \leq \underline{p}$, $\forall k = 1,\cdots,q$: Because $\pi(S_{D^{(q)}}^k) \leq \underline{p} < \pi(N), \forall k = 1,\cdots,q$, $\exists k' \in \{1,\cdots,q\} : \pi(\cup_{k=1}^{k'-1} S_{D^{(q)}}^k) \leq \underline{p} < \pi(\cup_{k=1}^{k'} S_{D^{(q)}}^k)$.

Define some dummy set $\tilde{S}$: $\pi\left(\left(\cup_{k=1}^{k'-1} S_{D^{(q)}}^k\right) \cup \tilde{S}\right) = \pi\left(\cup_{k=1}^{k'-1} S_{D^{(q)}}^k\right) + \pi\left(\tilde{S}\right) = \underline{p}$, and let $S' \equiv \left(\cup_{k=1}^{k'-1} S_{D^{(q)}}^k\right) \cup \tilde{S}$. By construction, $\pi(S') = \underline{p} \Rightarrow T_D^*(S') = 1$, and we can write:

$$T_{I^{(1)}} = 1 \ = \ T_D^*(S') \ \leq \ T_D^*(\tilde{S}) + \sum_{k=1}^{k'-1} T_D^*(S_{D^{(q)}}^k) \ < \ \sum_{k=1}^{k'} T_D^*(S_{D^{(q)}}^k) \ \leq \ \sum_{k=1}^{q} T_D^*(S_{D^{(q)}}^k),$$

where the first two equalities follow by Property 2, the first inequality follows because $T_D^*(S)$ is subadditive in $\pi(S)$, the second inequality follows because $T_D^*(S)$ is strictly increasing in $\pi(S)$, and the last inequality follows because $k' \leq q$. Hence $I^{(1)} \preceq D^{(q)}$, $q = 2,\cdots,n$.

In each case the optimal design class is $I^{(1)}$, completing the proof. □

Proof of Theorem 3. Consider the highest-difference composite cost function in $\tilde{C}$, that is, $\lambda = 1$ and $c(s) = \gamma \times s \Rightarrow \tilde{c}(s) = \gamma \times s, \forall s \in Z^+$, where the objective reduces to the minimization of $\sum_{k=1}^{q} s^k \times T^*(S^k)$.

First, we show that if $\pi(N) \leq \underline{p}$, then $D^{(n)} \preceq D^{(n-1)} \preceq \cdots \preceq D^{(1)}$. Consider a $D^{(q)}$ design, with partition $\boldsymbol{S}_{D^{(q)}}$, for any $q = 1, \cdots, n-1$. Then, $\exists k' \in \{1, \cdots, q\} : s^{k'}_{D^{(q)}} \geq 2$. We construct a feasible $D^{(q+1)}$ design by splitting set $S^{k'}_{D^{(q)}}$ into mutually exclusive sets, $S^{k'_1}$ and $S^{k'_2} : \cup_{r=1}^{2} S^{k'_r} = S^{k'}_{D^{(q)}}$ and $\cap_{r=1}^{2} S^{k'_r} = \emptyset$. Then:

$$\sum_{r=1}^{2} s^{k'_r} \times T^*_D(S^{k'_r}) \leq \sum_{r=1}^{2} s^{k'_r} \times T^*_D(S^{k'}_{D^{(q)}}) = s^{k'}_{D^{(q)}} \times T^*_D(S^{k'}_{D^{(q)}}),$$

where the inequality follows because, by construction $\pi(S^{k'_r}) \leq \pi(S^{k'}_{D^{(q)}}), r = 1, 2$, and $\sum_{r=1}^{2} s^{k'_r} = s^{k'}_{D^{(q)}}$, and $T^*_D(\pi(S))$ is increasing in $\pi(S) \in [0, \underline{p}]$ (Property 2). Because this result holds for a feasible $D^{(q+1)}$ design, it must hold for an optimal $D^{(q+1)}$ design, and we have that $D^{(q+1)} \preceq D^{(q)}$, $\forall q = 1, \cdots, n-1$.
We are ready to characterize an optimal design class.

<u>*Part 1.*</u> Case where $\pi_1 \leq \underline{p}$:

(a) Case where $\pi(N) \leq \underline{p}$: By Theorem 1, the optimal design class is $D^{(q)}$ for some $q = 1, \cdots, n$. Further, as shown above, $D^{(n)} \preceq D^{(n-1)} \preceq \cdots \preceq D^{(1)}$, hence the optimal design class is $D^{(n)}$.

(b) Case where $\pi(N) > \underline{p}$: By Theorem 1, the optimal design class is either $I^{(1)}$, or $D^{(q)}$ for some $q = 2, \cdots, n$, or $M^{(q)}$ for some $q = 2, \cdots, n-1$. From Eq. (7), $\pi_1 \leq \underline{p} \Rightarrow \pi_i \leq \underline{p}, \forall i \in N \Rightarrow T^*_D(\{i\}) \leq 1$, $\forall i \in N$. Further, $T^*_D(\pi(S))$ is increasing in $\pi(S) \in [0, \underline{p}]$, with $T^*_D(\underline{p}) = 1$, hence $T^*_D(\{i\}) \leq T^*_D(S), \forall i \in S, S \subseteq N$. Then:

$$T^*_D(\{i\}) \leq \min\{1, T^*_D(S)\}, \forall i \in S, S \subseteq N \Rightarrow \sum_{i \in S} T^*_D(\{i\}) \leq s \times \min\{1, T^*_D(S)\} \leq s \times T^*_D(S), \forall S \subseteq N, \quad (45)$$

and the result that $D^{(n)} \preceq D^{(q)}$, for any $q = 1, \cdots, n-1$, follows. Also, from Eq. (45), $TC_{D^{(n)}} = \sum_{i \in N} 1 \times T^*_D(\{i\}) \leq n = TC_{I^{(1)}}$. Hence, $D^{(n)} \preceq I^{(1)}$.

Finally, in an $M^{(q)}$ design, $T(S^{k'}_{M^{(q)}}, t^{k'}_{M^{(q)}} = 1) = 1$ for some $k' \in \{1, \cdots, q\}$, hence, by Eq. (45), $D^{(n)} \preceq M^{(q)}$, for any $q = 2, \cdots, n-1$. Hence, the optimal design class is $D^{(n)}$.

<u>*Part 2.*</u> Case where $\pi_n \geq \underline{p}$: By Theorem 1, the optimal design class is $I^{(1)}$. Next we show that the total cost of *any* individual-testing design $I^{(q)}, q = 2, \cdots, n$, is the same:

$$TC_{I^{(q)}} = \sum_{k=1}^{q} s^k_{I^{(q)}} \times T(S^k_{I^{(q)}}, 1) = \sum_{k=1}^{q} s^k_{I^{(q)}} = n = n \times T(N, 1) = TC_{I^{(1)}}.$$

Hence any individual-testing design, $I^{(q)}, q = 1, \cdots, n$, is optimal.

<u>*Part 3.*</u> Case where $\exists i \in N : \pi_{i+1} < \underline{p} < \pi_i$: By Theorem 1, the optimal design class is either $I^{(1)}$, or $M^{(q)}$ for some $q = 2, \cdots, n-i+1$. Decompose set N into two mutually exclusive and exhaustive sets, $S^1 = \{1, \cdots, i\}$ and $S^2 = \{i+1, \cdots, n\}$, that is, $\pi_j > \underline{p}, \forall j \in S^1$, and $\pi_j \leq \underline{p}, \forall j \in S^2$. Then, by part 2 of this theorem, any $I^{(q)}, q = 1, \cdots, i$, is optimal for set $S^1 = \{1, \cdots, i\}$; and by part 1 of this theorem, $D^{(n-i)}$ is optimal for set $S^2 = \{i+1, \cdots, n\}$. Because the total cost is additive over assay sets, an optimal design is the combination of the optimal designs for sets S^1 and S^2, that is, any $M^{(q)}$ for $q = n-i+1, \cdots, n$. □

Proof of Theorem 4. Because $\pi(N) \leq \underline{p}$, by Theorem 1, the optimal design class is $D^{(q)}$, with partition $\boldsymbol{S}_{D^{(q)}}$ for some $q = 1, \cdots, n$. Because $T^*_D(S)$ is subadditive in $\pi(S) \in [0, \underline{p}]$, we have that:

$$T_{D^{(1)}} = T^*_D(N) \leq \sum_{k=1}^{q} T^*_D(S^k_{D^{(q)}}), \forall q = 2, \cdots, n. \quad (46)$$

Recall that an optimal pool size $t^*(\pi(S))$ depends only on $\pi(S)$ (Property 2), that is, it does not change with λ; and $T^*_D(S)$ denotes the expected tests for set $S \subseteq N$ at the optimal pool size for set S. The proof is three-fold: First we show that $\exists \overline{\lambda}^{(1)} \leq 1$ such that $D^{(1)}(\lambda)$ is optimal if and only if $\lambda \leq \overline{\lambda}^{(1)}$, then we show that $\exists \overline{\lambda}^{(n-1)} \leq 1$ such that $D^{(n)}(\lambda)$ is optimal if and only if $\lambda > \max\left\{\overline{\lambda}^{(1)}, \overline{\lambda}^{(n-1)}\right\}$; finally we show that $\exists \overline{\lambda}^{(q)} \leq 1, q = 2, \cdots, n-1$, such that $D^{(r)}(\lambda)$, for some $r = 2, \cdots, q$, is optimal if and only if $\lambda \in \left(\max\left\{\overline{\lambda}^{(1)}, \overline{\lambda}^{(q-1)}\right\}, \overline{\lambda}^{(q)}\right]$.

We first prove that $\exists \overline{\lambda}^{(1)}: \;\; D^{(1)}(\lambda) \preceq D^{(q)}(\lambda), \forall q = 2, \cdots, n \Leftrightarrow \lambda \leq \overline{\lambda}^{(1)}$.

(a) We first show that, if for some λ, $D^{(1)}(\lambda) \preceq D^{(q)}(\lambda), \forall q = 2, \cdots, n$, then $D^{(1)}(\lambda - \epsilon) \preceq D^{(q)}(\lambda - \epsilon), \forall \epsilon \in [0, \lambda]$:

$$D^{(1)}(\lambda) \preceq D^{(q)}(\lambda), \forall q = 2, \cdots, n$$

$$\Leftrightarrow \tilde{c}(n) \times T_D^*(N) \leq \sum_{k=1}^{q} \tilde{c}(s_{D^{(q)}}^k) \times T_D^*(S_{D^{(q)}}^k), \forall q = 2, \cdots, n$$

$$\Leftrightarrow [\lambda \; c(n) + 1 - \lambda] \times T_D^*(N) \leq \sum_{k=1}^{q} [\lambda \; c(s_{D^{(q)}}^k) + 1 - \lambda] \times T_D^*(S_{D^{(q)}}^k), \forall q = 2, \cdots, n. \tag{47}$$

There are two possible cases:

(i) Case where $c(n) \times T_D^*(N) \leq \sum_{k=1}^{q} c(s_{D^{(q)}}^k) \times T_D^*(S_{D^{(q)}}^k), \forall q = 2, \cdots, n$: Then, the result trivially follows by (46) and the condition stated for this case, because we have, $\forall q = 2, \cdots, n$, and any $\epsilon \in [0, \lambda]$:

$$(\lambda - \epsilon) \; c(n) \times T_D^*(N) \leq (\lambda - \epsilon) \sum_{k=1}^{q} c(s_{D^{(q)}}^k) \times T_D^*(S_{D^{(q)}}^k), \quad \text{and} \quad [1 - (\lambda - \epsilon)] \times T_D^*(N) \leq [1 - (\lambda - \epsilon)] \times \sum_{k=1}^{q} T_D^*(S_{D^{(q)}}^k)$$

$$\Rightarrow TC_{D^{(1)}}(\lambda - \epsilon) \leq TC_{D^{(q)}}(\lambda - \epsilon).$$

(ii) Case where $c(n) \times T_D^*(N) > \sum_{k=1}^{q} c(s_{D^{(q)}}^k) \times T_D^*(S_{D^{(q)}}^k)$, for some $q = 2, \cdots, n$: It is sufficient to show that,

$$-\epsilon \; c(n) \times T_D^*(N) + \epsilon \times T_D^*(N) \leq -\epsilon \sum_{k=1}^{q} c(s_{D^{(q)}}^k) \times T_D^*(S_{D^{(q)}}^k) + \epsilon \; \sum_{k=1}^{q} T_D^*(S_{D^{(q)}}^k),$$

which holds, because by (46) and the condition stated for this case, we have that, for any $\epsilon \in [0, \lambda]$:

$$-\epsilon \; c(n) \times T_D^*(N) < -\epsilon \sum_{k=1}^{q} c(s_{D^{(q)}}^k) \times T_D^*(S_{D^{(q)}}^k), \quad \text{and} \quad \epsilon \times T_D^*(N) \leq \epsilon \; \sum_{k=1}^{q} T_D^*(S_{D^{(q)}}^k) \;\; \Rightarrow \;\; TC_{D^{(1)}}(\lambda - \epsilon) \leq TC_{D^{(q)}}(\lambda - \epsilon).$$

Thus, in both cases, $D^{(1)}(\lambda - \epsilon) \preceq D^{(q)}(\lambda - \epsilon), \forall q = 2, \cdots, n, \forall \epsilon \in [0, \lambda]$.

(b) To complete the proof, we now show that, if for some λ, $D^{(q)}(\lambda) \preceq D^{(1)}(\lambda)$, for some $q = 2, \cdots, n$, then $D^{(q)}(\lambda + \epsilon) \preceq D^{(1)}(\lambda + \epsilon), \forall \epsilon \in [0, 1 - \lambda]$. It is sufficient to show that,

$$\epsilon \; c(n) \times T_D^*(N) - \epsilon \times T_D^*(N) \geq \epsilon \; \sum_{k=1}^{q} c(s_{D^{(q)}}^k) \times T_D^*(S_{D^{(q)}}^k) - \epsilon \; \sum_{k=1}^{q} T_D^*(S_{D^{(q)}}^k).$$

Because $D^{(q)}(\lambda) \preceq D^{(1)}(\lambda)$ and by the inequality in (46), it must be true that,

$$c(n) \times T_D^*(N) \geq \sum_{k=1}^{q} c(s_{D^{(q)}}^k) \times T_D^*(S_{D^{(q)}}^k).$$

Then, for any $\epsilon \in [0, 1 - \lambda]$:

$$\epsilon \sum_{k=1}^{q} c(s_{D^{(q)}}^k) \times T_D^*(S_{D^{(q)}}^k) \leq \epsilon \; c(n) \times T_D^*(N), \quad \text{and} \quad -\epsilon \sum_{k=1}^{q} T_D^*(S_{D^{(q)}}^k) \leq -\epsilon \times T_D^*(N) \;\; \Rightarrow \;\; TC_{D^{(q)}}(\lambda + \epsilon) \leq TC_{D^{(1)}}(\lambda + \epsilon).$$

That is, $D^{(q)}(\lambda + \epsilon) \preceq D^{(1)}(\lambda + \epsilon), \forall \epsilon \in [0, 1 - \lambda]$, and the result follows.

Next, we prove that $\exists \overline{\lambda}^{(n-1)}: \; D^{(n)} \preceq D^{(q)}, \forall q = 1, \cdots, n-1 \Leftrightarrow \lambda > \max\left\{\overline{\lambda}^{(1)}, \overline{\lambda}^{(n-1)}\right\}$.

(a) We first show that, if for some λ, $D^{(q)}(\lambda) \preceq D^{(n)}(\lambda)$, for some $q = 1, \cdots, n-1$, then $D^{(q)}(\lambda - \epsilon) \preceq D^{(n)}(\lambda - \epsilon), \forall \epsilon \in [0, \lambda]$:

$$D^{(q)}(\lambda) \preceq D^{(n)}(\lambda)$$

$$\Leftrightarrow \sum_{k=1}^{q} \tilde{c}(s_{D^{(q)}}^k) \times T_D^*(S_{D^{(q)}}^k) \leq \sum_{k=1}^{n} \tilde{c}(1) \times T_D^*(\{k\})$$

$$\Leftrightarrow \sum_{k=1}^{q} [\lambda \; c(s_{D^{(q)}}^k) + 1 - \lambda] \times T_D^*(S_{D^{(q)}}^k) \leq \sum_{k=1}^{n} [\lambda \; c(1) + 1 - \lambda] \times T_D^*(\{k\}). \tag{48}$$

There are two possible cases:

(i) Case where $\sum_{k=1}^{q} c(s_{D^{(q)}}^k) \times T_D^*(S_{D^{(q)}}^k) \leq \sum_{k=1}^{n} c(1) \times T_D^*(\{k\})$: Then, the result trivially follows by (46)

and the condition stated for this case, because for any $\epsilon \in [0, \lambda]$:

$$(\lambda-\epsilon)\sum_{k=1}^{q} c(s^k_{D^{(q)}}) \times T^*_D(S^k_{D^{(q)}}) \leq (\lambda-\epsilon)\sum_{k=1}^{n} c(1) \times T^*_D(\{k\}), \quad \text{and} \quad (1-(\lambda-\epsilon))\sum_{k=1}^{q} T^*_D(S^k_{D^{(q)}}) \leq (1-(\lambda-\epsilon))\sum_{k=1}^{n} T^*_D(\{k\})$$

$$\Rightarrow TC_{D^{(q)}}(\lambda-\epsilon) \leq TC_{D^{(n)}}(\lambda-\epsilon).$$

(ii) Case where $\sum_{k=1}^{q} c(s^k_{D^{(q)}}) \times T^*_D(S^k_{D^{(q)}}) > \sum_{k=1}^{n} c(1) \times T^*_D(\{k\})$: It is sufficient to show that,

$$-\epsilon \sum_{k=1}^{n} c(1) \times T^*_D(\{k\}) + \epsilon \sum_{k=1}^{n} T^*_D(\{k\}) \leq -\epsilon \sum_{k=1}^{q} c(s^k_{D^{(q)}}) \times T^*_D(S^k_{D^{(q)}}) + \epsilon \sum_{k=1}^{q} T^*_D(S^k_{D^{(q)}}),$$

which holds, because by (46) and the condition stated for this case, we have that, for any $\epsilon \in [0, \lambda]$:

$$-\epsilon\sum_{k=1}^{q} c(s^k_{D^{(q)}}) \times T^*_D(S^k_{D^{(q)}}) < -\epsilon\sum_{k=1}^{n} c(1) \times T^*_D(\{k\}), \quad \text{and} \quad \epsilon\sum_{k=1}^{q} T^*_D(S^k_{D^{(q)}}) \leq \epsilon\sum_{k=1}^{n} T^*_D(\{k\})$$

$$\Rightarrow TC_{D^{(q)}}(\lambda-\epsilon) \leq TC_{D^{(n)}}(\lambda-\epsilon).$$

Thus, in both cases, $D^{(q)}(\lambda-\epsilon) \preceq D^{(n)}(\lambda-\epsilon), \forall \epsilon \in [0, \lambda]$.

(b) To complete the proof, we now show that, if for some λ, $D^{(n)}(\lambda) \preceq D^{(q)}(\lambda), \forall q = 1, \cdots, n-1$, then $D^{(n)}(\lambda+\epsilon) \preceq D^{(q)}(\lambda+\epsilon), \forall \epsilon \in [0, 1-\lambda]$. It is sufficient to show that,

$$\epsilon \sum_{k=1}^{q} c(s^k_{D^{(q)}}) \times T^*_D(S^k_{D^{(q)}}) - \epsilon \sum_{k=1}^{q} T^*_D(S^k_{D^{(q)}}) \geq \epsilon \sum_{k=1}^{n} c(1) \times T^*_D(\{k\}) - \epsilon \sum_{k=1}^{n} T^*_D(\{k\}).$$

Because of the optimality of $D^{(n)}(\lambda)$ and the inequality in (46), it must be true that,

$$\sum_{k=1}^{n} c(1) \times T^*_D(\{k\}) \leq \sum_{k=1}^{q} c(s^k_{D^{(q)}}) \times T^*_D(S^k_{D^{(q)}}).$$

Then, for any $\epsilon \in [0, 1-\lambda]$:

$$\epsilon\sum_{k=1}^{n} c(1) \times T^*_D(\{k\}) \leq \epsilon\sum_{k=1}^{q} c(s^k_{D^{(q)}}) \times T^*_D(S^k_{D^{(q)}}), \quad \text{and} \quad -\epsilon\sum_{k=1}^{n} T^*_D(\{k\}) \leq -\epsilon\sum_{k=1}^{q} T^*_D(S^k_{D^{(q)}})$$

$$\Rightarrow TC_{D^{(n)}}(\lambda+\epsilon) \leq TC_{D^{(q)}}(\lambda+\epsilon).$$

That is, $D^{(n)}(\lambda+\epsilon) \preceq D^{(q)}(\lambda+\epsilon), q = 1, \cdots, n-1, \forall \epsilon \in [0, 1-\lambda]$, and the result follows.

Finally, we show that $\exists \overline{\lambda}^{(q)} \leq 1, q = 2, \cdots, n-1$, such that if $\lambda \in \left(\max\left\{\overline{\lambda}^{(1)}, \overline{\lambda}^{(q-1)}\right\}, \overline{\lambda}^{(q)}\right]$, then the optimal solution is $D^{(r)}(\lambda)$, for some $r = 2, \cdots, q$. To this end, we first show that $\exists \lambda^q \leq 1, q = 3, \cdots, n$, such that $D^{(q-1)}(\lambda) \preceq D^{(q)}(\lambda), \forall \lambda \leq \lambda^q$.

Consider any $D^{(q)}$ design for $q \geq 2$. We construct a feasible $D^{(q-1)}$ design by combining any two assay sets, $k_1, k_2 \in \{1, \cdots, q\} : k_1 \neq k_2$, in the $D^{(q)}$ design, while keeping all remaining assays intact. We show that, if for some λ, $D^{(q-1)}(\lambda) \preceq D^{(q)}(\lambda)$, then $D^{(q-1)}(\lambda-\epsilon) \preceq D^{(q)}(\lambda-\epsilon), \forall \epsilon \in [0, \lambda]$. Because all assays other than k_1 and k_2 remain the same, it is sufficient to show that, given:

$$[\lambda\ c(s^{k_1} + s^{k_2}) + 1 - \lambda] \times T^*_D(\pi(S^{k_1} \cup S^{k_2})) \leq \sum_{r=1}^{2} [\lambda\ c(s^{k_r}) + 1 - \lambda]\ \times\ T^*_D(\pi(S^{k_r})), \tag{49}$$

the following inequality holds:

$$[(\lambda-\epsilon)\ c(s^{k_1} + s^{k_2}) + 1 - (\lambda-\epsilon)] \times T^*_D(\pi(S^{k_1} \cup S^{k_2})) \leq \sum_{r=1}^{2} [(\lambda-\epsilon)\ c(s^{k_r}) + 1 - (\lambda-\epsilon)] \times\ T^*_D(\pi(S^{k_r})), \tag{50}$$

equivalently, the following inequality holds:

$$\big(\epsilon c(s^{k_1}+s^{k_2})-\epsilon\big)\times T_D^*(\pi(S^{k_1}\cup S^{k_2}))\geq \sum_{r=1}^{2}\big(\epsilon c(s^{k_r})-\epsilon\big)\times\ T_D^*(\pi(S^{k_r})). \tag{51}$$

Because $T_D^*(\pi(S))$ is subadditive in $\pi(S)$, and $\pi(S)$ is subadditive in set S (Property A.1), for any $k_1,k_2\in\{1,\cdots,q\}: k_1\neq k_2$:

$$\pi(S^{k_1})+\pi(S^{k_2})\geq \pi(S^{k_1}\cup S^{k_2}),\text{ and} \tag{52}$$

$$\sum_{r=1}^{2}T_D^*(\pi(S^{k_r}))\geq T_D^*(\sum_{r=1}^{2}\pi(S^{k_r}))\geq T_D^*(\pi(S^{k_1}\cup S^{k_2})). \tag{53}$$

There are two possible cases:

(i) Case where $c(s^{k_1}+s^{k_2})\times T_D^*(\pi(S^{k_1}\cup S^{k_2}))\geq\sum_{r=1}^{2}c(s^{k_r})\times\ T_D^*(\pi(S^{k_r}))$: Then, for any $\epsilon>0$:

$$\epsilon\ c(s^{k_1}+s^{k_2})\times T_D^*(\pi(S^{k_1}\cup S^{k_2}))\geq \epsilon\ \sum_{r=1}^{2}c(s^{k_r})\times\ T_D^*(\pi(S^{k_r})), \tag{54}$$

and from Eq. (53),

$$-\epsilon\ T_D^*(\pi(S^{k_1}\cup S^{k_2}))\geq -\epsilon\ \sum_{r=1}^{2}T_D^*(\pi(S^{k_r})), \tag{55}$$

hence adding (54) and (55) leads to:

$$\Big(\epsilon c(s^{k_1}+s^{k_2})-\epsilon\Big)\times T_D^*(\pi(S^{k_1}\cup S^{k_2}))\geq \sum_{r=1}^{2}\Big(\epsilon c(s^{k_r})-\epsilon\Big)\times\ T_D^*(\pi(S^{k_r})), \tag{56}$$

that is, (51) is satisfied, and this feasible $D^{(q-1)}$ design dominates the optimal $D^{(q)}$ design, and this result continues to hold for the optimal $D^{(q-1)}$ design, that is, $D^{(q-1)}(\lambda-\epsilon)\preceq D^{(q)}(\lambda-\epsilon),\forall\epsilon\in[0,\lambda]$.

(ii) Case where $c(s^{k_1}+s^{k_2})\times T_D^*(\pi(S^{k_1}\cup S^{k_2}))<\sum_{r=1}^{2}c(s^{k_r})\times\ T_D^*(\pi(S^{k_r}))$: Then, we have that

$$\Big(\lambda c(s^{k_1}+s^{k_2})+1-\lambda\Big)\times T_D^*(\pi(S^{k_1}\cup S^{k_2}))<\sum_{r=1}^{2}\Big(\lambda c(s^{k_r})+1-\lambda\Big)\times\ T_D^*(\pi(S^{k_r})),\ \forall\lambda\in[0,1], \tag{57}$$

that is, $D^{(q-1)}(\lambda)\preceq D^{(q)}(\lambda),\forall\lambda\in[0,1]$.

Then, it follows that $\exists\lambda^q\leq 1, q=3,\cdots,n$, such that $D^{(q-1)}\preceq D^{(q)},\forall\lambda\leq\lambda^q$. Therefore, the result follows by setting $\overline{\lambda}^{(q-1)}=\min_{k=q,\cdots,n}\{\lambda^k\}$, for $q=3,\cdots,n$.

Note that only the existence of the $D^{(1)}$ optimal-region, $\lambda\in[0,\overline{\lambda}^{(1)}]$, is guaranteed. In other words, $D^{(n)}$-optimal region may be empty if $\max\left\{\overline{\lambda}^{(1)},\overline{\lambda}^{(n-1)}\right\}=1\Rightarrow\left(\max\left\{\overline{\lambda}^{(1)},\overline{\lambda}^{(n-1)}\right\},1\right]=\emptyset$; and the region where $D^{(r)}$, for some $r=1,\cdots,q$, is optimal may be empty if $\overline{\lambda}^{(q)}\leq\overline{\lambda}^{(1)}\Rightarrow\left(\max\left\{\overline{\lambda}^{(1)},\overline{\lambda}^{(q-1)}\right\},\overline{\lambda}^{(q)}\right]=\emptyset$. □

Proof of Lemma 1. From Theorem 4, $D^{(n)}\preceq D^{(q)},\forall q=1,\cdots,n-1\Leftrightarrow\lambda>\max\left\{\overline{\lambda}^{(1)},\overline{\lambda}^{(n-1)}\right\}$, equivalently,

$$\sum_{k=1}^{n}\tilde{c}(1,\lambda)\times T_D^*\big(\pi(\{k\};\boldsymbol{p})\big)\leq\sum_{k=1}^{q}\tilde{c}(s^k_{D^{(q)}},\lambda)\times T_D^*\big(\pi(S^k_{D^{(q)}};\boldsymbol{p})\big),\quad\forall q=1,\cdots,n-1\Leftrightarrow\lambda>\max\left\{\overline{\lambda}^{(1)},\overline{\lambda}^{(n-1)}\right\}. \tag{58}$$

By Definition 6, for any $\boldsymbol{p}'$ that is more correlated than $\boldsymbol{p}$, $\pi(S;\boldsymbol{p}')\leq\pi(S;\boldsymbol{p}),\forall S\subseteq N: s\geq 2$, and $\pi(S;\boldsymbol{p}')=\pi(S;\boldsymbol{p}),\forall S\subseteq N: s=1$, and $T_D^*(\pi(S))$ is increasing in $\pi(S)\in[0,\underline{p}]$ (Property 2), that is, as $\boldsymbol{p}$ becomes more correlated, for each given λ, the LHS of (58) remains the same, while the RHS is non-increasing. Therefore, the range of λ values for which the condition in (58) is satisfied either remains the same or shrinks. Then due to the $D^{(n)}$-optimality region delineated in Theorem 4, $\overline{\lambda}^{(n-1)}$ must be non-decreasing as $\boldsymbol{p}$ gets more correlated. □

Proof of Theorem 5.

Part 1. We first prove the result for the Dorfman design class.

(a) Independent diseases case: Consider that the prevalences of the diseases in set N are mutually independent. Consider any assay $S \subseteq N$, and swap any two diseases $i \in S,\ j \in N \backslash S$. The difference between the prevalences of the new assay $S\backslash\{i\} \cup \{j\}$, and the original assay S, can be expressed as follows:

$$\pi(S\backslash\{i\} \cup \{j\}) - \pi(S) = \frac{(\pi_j - \pi_i)[1 - \pi(S)]}{1 - \pi_i} = (\pi_j - \pi_i) \prod_{r \in S\backslash\{i\}} (1 - \pi_r). \tag{59}$$

Consider any unordered partition $\boldsymbol{S} = (S^1, S^2)$, where $S^1 = \{i_1, \cdots, i_{s^1}\}$ and $S^2 = \{j_1, \cdots, j_{s^2}\}$, with cardinality vector $\boldsymbol{s} = (s^1, s^2)$, where the diseases in each set $S^k, k = 1, 2$, are arranged following a non-increasing order of disease prevalences, that is, $\pi_{i_1} \geq \pi_{i_2} \geq \cdots \geq \pi_{i_{s^1}}$ and $\pi_{j_1} \geq \pi_{j_2} \geq \cdots \geq \pi_{j_{s^2}}$. Because $\mathbf{S}$ is not an ordered partition, we must have that, either $\pi_{i_1} > \pi_{j_1} > \pi_{i_{s^1}}$, or $\pi_{j_1} > \pi_{i_1} > \pi_{j_{s^2}}$, and in both cases, it follows that:

$$\pi_{i_1} > \pi_{j_{s^2}} \text{ and } \pi_{j_1} > \pi_{i_{s^1}}. \tag{60}$$

For each disease $r \in N$, define two dummy diseases, $r^{(\epsilon)}$ and $r^{(-\epsilon)}$, with respective prevalences, $\pi_{r^{(\epsilon)}} = \pi_r + \epsilon$ and $\pi_{r^{(-\epsilon)}} = \pi_r - \epsilon$, for any $\epsilon > 0$ such that $\pi_{r^{(\epsilon)}}, \pi_{r^{(-\epsilon)}} \in [0, 1]$. Then, from Eq. (59), we have that:

$$\pi(S^1\backslash\{i_{s^1}\} \cup \{{i_{s^1}}^{(\epsilon)}\}) - \pi(S^1) = \epsilon \prod_{r \in S^1\backslash\{i_{s^1}\}} (1 - \pi_r) \ \leq \ \epsilon \prod_{r \in S^1\backslash\{i_1\}} (1 - \pi_r) = \pi(S^1) - \pi(S^1\backslash\{i_1\} \cup \{{i_1}^{(-\epsilon)}\}), \tag{61}$$

and a similar inequality can be derived for set S^2.

In the following, we show that swapping diseases between sets S^1 and S^2, as many times as needed so that set S^1 ends up containing either the s^1 highest prevalence diseases, or the s^1 lowest prevalence diseases in set N (depending on the cost structure), and hence S^2 ends up containing the remaining diseases, either reduces, or does not change, the total cost. Thus we conclude that there exists an optimal partition that is ordered. There are two possible cases:

(i) Case where Condition **(C1)** holds, where

Condition **(C1):** $\tilde{c}(s^1) \times [T^*_D(S^1\backslash\{i_{s^1}\} \cup \{{i_{s^1}}^{(\epsilon)}\}) - T^*_D(S^1)] \geq \tilde{c}(s^2) \times [T^*_D(S^2) - T^*_D(S^2\backslash\{j_1\} \cup \{{j_1}^{(-\epsilon)}\})]$.

Then, by Eq. (61), because $T^*_D(S)$ is concave increasing in any $\pi_r, r \in S$ (Property A.2), we have that:

$$T^*_D(S^1\backslash\{i_{s^1}\} \cup \{{i_{s^1}}^{(\epsilon)}\}) - T^*_D(S^1) \leq T^*_D(S^1) - T^*_D(S^1\backslash\{i_1\} \cup \{{i_1}^{(-\epsilon)}\}). \tag{62}$$

Similarly,

$$T^*_D(S^2\backslash\{j_{s^2}\} \cup \{{j_{s^2}}^{(\epsilon)}\}) - T^*_D(S^2) \leq T^*_D(S^2) - T^*_D(S^2\backslash\{j_1\} \cup \{{j_1}^{(-\epsilon)}\}). \tag{63}$$

Then, Condition **(C1)** and Eqs. (62)-(63) lead to:

$$\begin{aligned}
\tilde{c}(s^1) \times [T^*_D(S^1) - T^*_D(S^1\backslash\{i_1\} \cup \{{i_1}^{(-\epsilon)}\})] \geq & \tilde{c}(s^1) \times [T^*_D(S^1\backslash\{i_{s^1}\} \cup \{{i_{s^1}}^{(\epsilon)}\}) - T^*_D(S^1)] \\
\geq & \tilde{c}(s^2) \times [T^*_D(S^2) - T^*_D(S^2\backslash\{j_1\} \cup \{{j_1}^{(-\epsilon)}\})] \\
\geq & \tilde{c}(s^2) \times [T^*_D(S^2\backslash\{j_{s^2}\} \cup \{{j_{s^2}}^{(\epsilon)}\}) - T^*_D(S^2)] \\
\Leftrightarrow \tilde{c}(s^1) \times T^*_D(S^1) + \tilde{c}(s^2) \times T^*_D(S^2) \geq & \tilde{c}(s^1) \times T^*_D(S^1\backslash\{i_1\} \cup \{{i_1}^{(-\epsilon)}\}) + \tilde{c}(s^2) \times T^*_D(S^2\backslash\{j_{s^2}\} \cup \{{j_{s^2}}^{(\epsilon)}\}),
\end{aligned}$$

and letting $\epsilon = \pi_{i_1} - \pi_{j_{s^2}} (> 0)$ yields a partition $\boldsymbol{S'} = (S^{1'}, S^{2'})$, where $S^{1'} = S^1\backslash\{i_1\} \cup \{j_{s^2}\}$ and $S^{2'} = S^2\backslash\{j_{s^2}\} \cup \{i_1\}$, and $TC(\boldsymbol{S'}, \boldsymbol{t^*}(\boldsymbol{S'})) \leq TC(\boldsymbol{S}, \boldsymbol{t^*}(\boldsymbol{S}))$.
Observe that by Eqs. (59)-(60), we have that:

$$\pi(S^{1'}) - \pi(S^1) = \pi(S^1\backslash\{i_1\} \cup \{j_{s^2}\}) - \pi(S^1) = (\pi_{j_{s^2}} - \pi_{i_1}) \frac{[1 - \pi(S^1)]}{1 - \pi_{i_1}} < 0 \ \Leftrightarrow \ \pi(S^{1'}) < \pi(S^1), \text{ and} \tag{64}$$

$$\pi(S^{2'}) - \pi(S^2) = \pi(S^2\backslash\{j_{s^2}\} \cup \{i_1\}) - \pi(S^2) = (\pi_{i_1} - \pi_{j_{s^2}}) \frac{[1 - \pi(S^2)]}{1 - \pi_{j_{s^2}}} > 0 \ \Leftrightarrow \ \pi(S^{2'}) > \pi(S^2). \tag{65}$$

Then, it is sufficient to show that if the new $\boldsymbol{S'} = (S^{1'}, S^{2'})$ is not an ordered partition, then Condition **(C1)** is still satisfied, and hence, starting with the new unordered partition $\boldsymbol{S'}$, one can swap the highest prevalence disease in set $S^{1'}$ with the lowest prevalence disease in set $S^{2'}$, in order to either reduce or keep the same total cost. Then one can repeat this argument to swap as many times as needed so that the revised set S^1 contains the s^1 lowest prevalence diseases, hence the revised set S^2 contains the s^2 highest prevalence diseases, resulting in an ordered

partition that is optimal.
To this end, we wish to show that:

$$\tilde{c}(s^1) \times [T_D^*(S^{1'} \backslash \{i_{s^1}\} \cup \{i_{s^1}{}^{(\epsilon)}\}) - T_D^*(S^{1'})] \geq \tilde{c}(s^2) \times [T_D^*(S^{2'}) - T_D^*(S^{2'} \backslash \{j_1\} \cup \{j_1{}^{(-\epsilon)}\})],$$

that is, Condition **(C1)** continues to be satisfied by the new partition $\boldsymbol{S'} = (S^{1'}, S^{2'})$.
By Eqs. (59), (64), and (65), we have that:

$$\pi(S^1 \backslash \{i_{s^1}\} \cup \{i_{s^1}{}^{(\epsilon)}\}) = \frac{\epsilon[1-\pi(S^1)]}{1-\pi_{i_{s^1}}} \quad < \quad \frac{\epsilon[1-\pi(S^{1'})]}{1-\pi_{i_{s^1}}} = \pi(S^{1'} \backslash \{i_{s^1}\} \cup \{i_{s^1}{}^{(\epsilon)}\}), \text{ and} \tag{66}$$

$$\pi(S^2) - \pi(S^2 \backslash \{j_1\} \cup \{j_1{}^{(-\epsilon)}\}) = \frac{\epsilon[1-\pi(S^2)]}{1-\pi_{j_1}} \quad > \quad \frac{\epsilon[1-\pi(S^{2'})]}{1-\pi_{j_1}} = \pi(S^{2'} - \pi(S^{2'} \backslash \{j_1\} \cup \{j_1{}^{(-\epsilon)}\}). \tag{67}$$

By Eqs. (66)-(67), and because $T_D^*(S)$ is concave increasing in any $\pi_r, r \in S$ (Property A.2), we can write:

$$T_D^*(S^{1'} \backslash \{i_{s^1}\} \cup \{i_{s^1}{}^{(\epsilon)}\}) - T_D^*(S^{1'}) \geq T_D^*(S^1 \backslash \{i_{s^1}\} \cup \{i_{s^1}{}^{(\epsilon)}\}) - T_D^*(S^1), \text{ and} \tag{68}$$

$$T_D^*(S^2) - T_D^*(S^2 \backslash \{j_1\} \cup \{j_1{}^{(-\epsilon)}\}) \geq T_D^*(S^{2'}) - T_D^*(S^{2'} \backslash \{j_1\} \cup \{j_1{}^{(-\epsilon)}\}), \tag{69}$$

and we have that:

$$\begin{aligned}
\tilde{c}(s^1) \times [T_D^*(S^{1'} \backslash \{i_{s^1}\} \cup \{i_{s^1}{}^{(\epsilon)}\}) - T_D^*(S^{1'})] \geq & \tilde{c}(s^1) \times [T_D^*(S^1 \backslash \{i_{s^1}\} \cup \{i_{s^1}{}^{(\epsilon)}\}) - T_D^*(S^1)] \\
\geq & \tilde{c}(s^2) \times [T_D^*(S^2) - T_D^*(S^2 \backslash \{j_1\} \cup \{j_1{}^{(-\epsilon)}\})] \\
\geq & \tilde{c}(s^2) \times [T_D^*(S^{2'}) - T_D^*(S^{2'} \backslash \{j_1\} \cup \{j_1{}^{(-\epsilon)}\})],
\end{aligned}$$

where the first and third inequalities follow by Eqs. (68)-(69), and the second inequality follows by Condition **(C1)**. That is, Condition **(C1)** continues to hold for partition $\boldsymbol{S'} = (S^{1'}, S^{2'})$.

Hence, if $\boldsymbol{S'}$ is an ordered partition, then we have attained an ordered partition $\boldsymbol{S'}$ for which $TC(\boldsymbol{S'}, \boldsymbol{t^*}(\boldsymbol{S'})) \leq TC(\boldsymbol{S}, \boldsymbol{t^*}(\boldsymbol{S}))$; and if $\boldsymbol{S'}$ is not an ordered partition, then we can repeat the same process by swapping the highest prevalence disease in set $S^{1'}$ with the lowest prevalence in set $S^{2'}$, thus either reducing or keeping the same total cost until an ordered partition in which the revised set S^1 contains the s^1 lowest prevalence diseases and the revised set S^2 contains the s^2 highest prevalence diseases is attained, establishing the desired result.

(ii) Case where Condition **(C1)** does not hold, that is,

$$\tilde{c}(s^1) \times [T_D^*(S^1 \backslash \{i_{s^1}\} \cup \{i_{s^1}{}^{(\epsilon)}\}) - T_D^*(S^1)] < \tilde{c}(s^2) \times [T_D^*(S^2) - T_D^*(S^2 \backslash \{j_1\} \cup \{j_1{}^{(-\epsilon)}\})]$$

The proof of this case follows similarly to the proof of Case 1, by repeatedly swapping the lowest prevalence disease in set S^1 with the highest prevalence disease in set S^2 (i.e., starting with swapping $i_{s^1} \in S^1$ with $j_1 \in S^2$) until an ordered partition in which the revised set S^1 contains the s^1 highest prevalence diseases and the revised set S^2 contains the s^2 lowest prevalence diseases is attained, and it can be shown that this ordered partition incurs a total cost that is less than or equal to the cost of the original partition.

(b) <u>No co-infections case:</u> Consider that the prevalences of the diseases in set N are mutually exclusive. Then, $\pi(S) = \sum_{i \in S} \pi_i, \forall S \subseteq N$ (Eq. (4)). Consider any $D^{(q)}$ design, $q = 2, \cdots, n$, with partition $\boldsymbol{S} = (S^k)_{k=1,\cdots,q}$, and a fixed cardinality vector $\boldsymbol{s} = (s^k)_{k=1,\cdots,q}$. Because the size of each assay, $s^k, k = 1, \cdots, q$, is fixed for all possible partitions, $\tilde{c}(s^k)$ becomes a constant, hence $\tilde{c}(s^k) \times T_D^*(S^k)$ is concave increasing in $\pi(S^k : |S^k| = s^k), k = 1, \cdots, q$ (Property 2), that is, considering all sets S^k with cardinality $|S^k| = s^k$. Hence, the total cost, $TC_{D^{(q)}}(\boldsymbol{S} : |\boldsymbol{S}| = \boldsymbol{s})$, is minimized by an ordered q-partition [3, 21].

<u>*Part 2.*</u> We next prove the result for the mixed-testing design class. Consider any $M^{(q)}$ design, $q = 2, \cdots, n$, and let N^I and N^D respectively denote the set of diseases that are tested (in any number of assays) individually, and via pooling, with respective cardinalities, $n^I, n^D \in Z^+ : n^I + n^D = n$. Then, $N^I \cup N^D = N$ and $N^I \cap N^D = \emptyset$. By Theorem 4, $M^{(q)}$ contains one assay, comprised of all diseases in set N^I, that is individually tested, and $q-1$ assays (i.e., subsets of set N^D) that are pooled.

Next, we turn our attention to those diseases that are tested via pooling, i.e., set N^D, and study the optimal partition of set N^D, which, by definition of $M^{(q)}$, needs to be a $q-1$-partition, with each assay using pooling. Let $\boldsymbol{S}(N^D) = (S^k(N^D))_{k=1,\cdots,q-1}$ denote any $q-1$-partition of set N^D. Then, by Eq. (19), we have that

$$TC(\boldsymbol{S}(N^D), \boldsymbol{t}_D^*(\boldsymbol{S}(N^D))) = \sum_{k=1}^{q-1} \tilde{c}(s^k(N^D)) \times T_D^*(S^k(N^D)) = \sum_{k=1}^{q-1} \tilde{c}(s^k(N^D)) \times \left[\frac{1}{t_D^*(S^k(N^D))} + 1 - \left(1 - \pi(S^k(N^D))\right)^{t_D^*(S^k(N^D))}\right].$$

First, we show that there exists an optimal $M^{(q)}$ design in which set N^I contains the n^I highest prevalence diseases in set N. Assume, to the contrary, that this is not the case. Then, $\exists i_1 \in N^I, i_2 \in N^D : \pi_{i_1} < \pi_{i_2}$. Assume, without loss of generality, that $i_2 \in S^{k'}(N^D) \subseteq N^D$, for some $k' = 1, \cdots, q-1$. Then, swapping diseases i_1 and i_2 will not affect the total cost of individual testing, because $TC(N^I, t=1) = TC(N^I \cup \{i_2\} \setminus \{i_1\}, 1) = \tilde{c}(n^I)$, but it will alter the total cost of pooled testing by $TC(\boldsymbol{S}(N^D \cup \{i_1\} \setminus \{i_2\})) - TC(\boldsymbol{S}(N^D)) = \tilde{c}(s^{k'}(N^D)) \times T_D^*(S^{k'}(N^D) \cup \{i_1\} \setminus \{i_2\})) - \tilde{c}(s^{k'}(N^D)) \times T_D^*(S^{k'}(N^D)) < 0$, where the inequality follows because $T_D^*(S)$ is strictly increasing in $\pi(S)$ (Property 2); and for both the independent diseases and no co-infections cases, $\pi(S)$ is linear increasing in π_i, $\forall i \in S$ (Eqs. (15)-(16)), hence, $\pi(S^{k'}(N^D)) > \pi(S^{k'}(N^D) \cup \{i_1\} \setminus \{i_2\})$, that is, we have identified a q-partitioned mixed-testing design with a lower total cost. Thus, there exists an optimal mixed-testing design in which set N^I contains $\{1, \cdots, n^I\}$. The second part of the result, that the diseases in set N^D are tested via pooling following an ordered $q-1$-partition, follows directly from part 1 of this theorem, completing the proof. □

Proof of Corollary 1.

Part 1. The reduction, of the problem of finding an optimal partition of set N, to a Shortest Path Problem follows from Theorem 5, which states the existence of an optimal design that uses an ordered partition under the assumptions stated in this corollary, in light of Lemma 1 in [21].

Part 2. The construction of graph $G(V(N), E(N))$ requires the computation of all edge weights, $w_{i,j} = \tilde{c}(s, \lambda) \times T^*(S)$, where $S = \{i, i+1, \cdots, j-1\}$, $\forall i, j \in V(N) : i < j$. Hence, $n+1-i$ computations are needed for each $w_{i,j}, i < j \leq n+1$, $i \in N$, for a total of $\sum_{i=1}^{n}(n+1-i) = \frac{1}{2}n(n+1)$ computations, leading to polynomial complexity in the order of $O(n^2)$. Solving the Shortest Path Problem by a topological sorting algorithm also has polynomial complexity, $O(n^2)$ [26], establishing the desired result. □

B Case Study: Details and Supporting Results

Assay cost function: Based on [76], which reports the per test cost for 2-plex and 20-plex PCR respiratory assays as \$42 and \$114.8, respectively, we explore various concave cost functions that satisfy, $21 \leq c(1) \leq 42$ and $c(20) = 114.8$. We consider a functional form that has a fixed cost per assay, and a variable cost per disease bundled. For the two extreme values for $c(1)$ (along with the given value of $c(20)$), at $c(1) = 21$, $c(s) = 16.06 + 4.94 \times s$; and at $c(1) = 42$, $c(s) = 38.17 + 3.83 \times s$. In §6, we provide the results for the $c(s) = 25.54 + 4.46 \times s$ function, yielding $c(1) = 30$; sensitivity analysis on cost parameters indicate similar qualitative findings.

CI for R-TD: For each year in the study period, we use the 52 weekly prevalence data for each disease to construct a 95% CI for each $\Pi_i, i \in N$, based on the Wald's method, e.g., [65], which utilizes the normal distribution approximation invoked by the Central Limit Theorem: $\hat{\pi}_i \pm \frac{z \times \hat{\sigma}_i}{\sqrt{52}}$, where $\hat{\pi}_i$ and $\hat{\sigma}_i$ respectively represent the average and standard deviation for the 52 weeks, and z is the corresponding standard normal CDF.

Price of robustness ratio (PoR) (%): $\frac{[TC^{\mathbf{R\text{-}TD}^*}(\lambda) - TC^{\mathbf{TD}^*}(\lambda)]}{TC^{\mathbf{TD}^*}(\lambda)} \times 100$.

Table B.1: PoR for 2018 and 2021 perfect-information **R-TD** designs, based on 2018 and 2021 data, resp.

λ range	0.00-0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00
2018-PoR (%)	0.000	0.232	0.020	0.022	0.024	0.026	0.037	1.600	2.395	1.203
2021-PoR (%)	0.000	0.000	0.000	-2.392	0.000	0.030	0.000	0.000	0.000	0.000

Table B.2: VoJ for 2018 **TD** designs (with and without seasonality) based on 2018 data - without COVID-19 testing

λ range	0-0.15	0.20-0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00
$VoJ(\lambda)$ (%) - Without seasonality	0.0	0.0	0.4	1.0	1.6	2.4	3.9	5.5	7.0	8.5	10.2	13.4	16.7	20.5
$VoJ(\lambda)$ (%) - With seasonality	5.3	5.2	5.4	6.2	7.2	8.3	9.7	11.3	13.0	14.6	16.7	19.4	22.2	25.2

Table B.3: The metrics for 2018 **TD** and **R-TD** designs based on 2019-2021 data - without COVID-19 testing

Model ($n = 17$)	Range of λ Values	Testing Cost Mean (Min-Max)	Number of Tests Mean (Min-Max)
TD	0.00-0.40	1.00 (1.00-1.00)	1.00 (1.00-1.00)
	0.45-0.55	0.93 (0.93-0.93)	1.05 (1.05-1.06)
	0.60-0.80	0.88 (0.83-1.07)	1.21 (1.11-1.62)
	0.85	0.86 (0.82-1.03)	1.25 (1.17-1.57)
	0.90	0.81 (0.74-0.94)	1.39 (1.26-1.63)
	0.95-1.00	0.79 (0.69-0.97)	1.56 (1.34-1.93)
R-TD	0.00-0.40	1.00 (1.00-1.00)	1.00 (1.00-1.00)
	0.45-0.55	0.93 (0.93-0.93)	1.05 (1.05-1.06)
	0.60-0.80	0.88 (0.83-1.06)	1.21 (1.11-1.59)
	0.85	0.86 (0.82-1.02)	1.25 (1.17-1.55)
	0.90	0.84 (0.79-0.98)	1.32 (1.22-1.63)
	0.95-1.00	0.82 (0.72-0.99)	1.54 (1.34-1.90)

Table B.4: The metrics for modified 2018 **TD** and **R-TD** designs based on 2021 data

Model ($n = 17$)	Modified 2018 design: Range of λ Values	Modified 2018 design: Testing Cost Mean (Min-Max)	Modified 2018 design: Number of Tests Mean (Min-Max)	2021 perfect-information design: Range of λ Values	2021 perfect-information design: Testing Cost Mean (Min-Max)	2021 perfect-information design: Number of Tests Mean (Min-Max)
Without COVID-19 Testing						
TD	0.00-0.40	0.76 (0.42-0.94)	0.76 (0.42-0.94)	0.00-0.35	0.76 (0.42-0.94)	0.76 (0.42-0.94)
	0.45-0.60	0.68 (0.37-1.03)	0.91 (0.49-1.60)	0.40-0.75	0.62 (0.36-0.77)	0.83 (0.49-1.02)
	0.65-0.85	0.63 (0.35-0.91)	0.94 (0.54-1.42)	0.80	0.60 (0.35-0.75)	0.90 (0.53-1.12)
	0.90-0.95	0.60 (0.36-0.93)	1.16 (0.69-1.89)	0.85-0.90	0.56 (0.34-0.75)	1.10 (0.67-1.47)
	1.00	0.60 (0.36-0.94)	1.35 (0.81-2.13)	0.95-1.00	0.56 (0.34-0.74)	1.12 (0.69-1.47)
R-TD	0.00-0.35	0.76 (0.42-0.94)	0.76 (0.42-0.94)	0.00-0.30	0.76 (0.42-0.94)	0.76 (0.42-0.94)
	0.40-0.55	0.68 (0.37-1.02)	0.90 (0.49-1.57)	0.35-0.70	0.62 (0.36-0.77)	0.83 (0.49-1.02)
	0.60-0.80	0.63 (0.36-0.90)	0.94 (0.54-1.40)	0.75	0.60 (0.35-0.75)	0.90 (0.53-1.12)
	0.85	0.62 (0.37-0.87)	0.98 (0.58-1.38)	0.80	0.57 (0.34-0.78)	1.07 (0.65-1.49)
	0.90	0.60 (0.36-0.92)	1.16 (0.69-1.87)	0.85	0.56 (0.34-0.75)	1.10 (0.67-1.47)
	0.95	0.59 (0.36-0.86)	1.25 (0.76-1.75)	0.90-1.00	0.56 (0.34-0.74)	1.12 (0.69-1.47)
	1.00	0.61 (0.37-0.93)	1.38 (0.85-2.13)			
With COVID-19 Testing						
TD	0.00-0.35	0.86 (0.59-1.02)	0.86 (0.59-1.02)	0.00-0.30	0.86 (0.59-1.02)	0.86 (0.59-1.02)
	0.40-0.55	0.77 (0.51-1.11)	1.01 (0.65-1.70)	0.35-0.70	0.71 (0.49-0.84)	0.93 (0.66-1.10)
	0.60-0.80	0.70 (0.48-0.98)	1.05 (0.72-1.53)	0.75	0.68 (0.48-0.82)	1.00 (0.71-1.21)
	0.85	0.66 (0.47-0.98)	1.27 (0.88-2.01)	0.80	0.63 (0.45-0.81)	1.21 (0.87-1.57)
	0.90	0.66 (0.46-0.97)	1.36 (0.93-2.02)	0.85-1.00	0.62 (0.45-0.80)	1.23 (0.89-1.57)
	0.95	0.65 (0.46-0.92)	1.36 (0.97-1.89)			
	1.00	0.66 (0.46-0.99)	1.46 (1.01-2.25)			
R-TD	0.00-0.35	1.00 (1.00-1.00)	1.00 (1.00-1.00)	0.00-0.30	0.86 (0.59-1.02)	0.86 (0.59-1.02)
	0.40-0.55	0.90 (0.86-1.12)	1.16 (1.06-1.70)	0.35-0.65	0.71 (0.49-0.84)	0.93 (0.66-1.10)
	0.60-0.85	0.81 (0.77-0.99)	1.20 (1.13-1.54)	0.70-0.75	0.68 (0.48-0.82)	1.00 (0.71-1.21)
	0.90	0.81 (0.76-1.04)	1.36 (1.24-1.93)	0.80-1.00	0.62 (0.45-0.80)	1.23 (0.89-1.57)
	0.95	0.65 (0.46-0.91)	1.36 (0.97-1.87)			
	1.00	0.66 (0.46-0.98)	1.49 (1.05-2.25)			